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PART I.

No. 1. — *List of Dredging Stations occupied by the United States Coast Survey Steamers "Corwin," "Bibb," "Hassler," and "Blake," from* 1867 *to* 1879. BENJAMIN PEIRCE *and* CARLILE P. PATTERSON, *Superintendents of the Coast Survey.*

THE following stations were occupied by the U. S. Coast Survey Steamer "Corwin," Acting-Master R. Platt, U. S. N., commanding, in 1867, in connection with a survey for a telegraph cable between Key West and Havana. The dredging operations were in charge of L. F. Pourtalès, Assist. U. S. Coast Survey. The expedition was cut short by the breaking out of yellow-fever on board.

Date.	Position.	Depth.	Locality.
May 17	1	90–100 fms.	5 m. S.S.W. of Sand Key, Fla.
" 24	2	270 "	1.6 m. from Chorrera, Cuba.
" 25	3	350 "	2 m. " "
" 29	4	270 "	1.6 m. " "

The dredgings in 1868 and 1869 were made on board the U. S. Coast Survey steamer "Bibb," Acting-Master R. Platt, U. S. N., commanding, by L. F. Pourtalès, Assist. U. S. Coast Survey. They are all comprised in the Florida Straits between Tortugas and Cape Florida. (See U. S. Coast Survey, General Coast Chart No. X., Coast Survey Report for 1850.)

1868.	No. of Dredging.	Fms.	Locality.
April 23	2d position	195	Off Sombrero.
" "	3d "	115	" "
May 1	7	111	" "
" "	6	121	" "
" "	5	111	" "
" "	4	152	" "
" "	3	183	" "
" "	2	262	" "
" "	1	517	" "
" 4	1	19	Off Bahia Honda.
" "	4	75	" "
" "	5	95	" "

1868.		No. of Dredging.	Fms.	Locality.	
May	4	6	105	Off Bahia Honda.	
"	"	7	100	"	"
"	"	9	119	"	"
"	"	10	128	"	"
"	"	11	176	"	"
"	"	12	324	"	"
"	"	13	418	"	"
"	6	1	16	Off American Shoal.	
"	"	3	43	"	"
"	"	4	55	"	"
"	"	5	75	"	"
"	"	6	83	"	"
"	"	7	98	"	"
"	"	8	94	"	"
"	"	9	100	"	"
"	8	1	111	"	"
"	"	3	150	"	"
"	"	4	135	"	"
"	"	5	266	"	"
"	9	2	34	Off the Samboes.	
"	"	4	67	"	"
"	"	5	80	"	"
"	"	6	93	"	"
"	"	7	96	"	"
"	"	8	101	"	"
"	"	9	106	"	"
"	"	10	106	"	"
"	"	11	116	"	"
"	"	12	123	"	"
"	"	13	125	"	"
"	"	14	125	"	"
"	"	16	139	"	"
"	"	17	147	"	"
"	"	18	298	"	"
"	"	19	237	"	"
"	11	2	26	Off Sand Key.	
"	"	3	54	"	"
"	"	4	67	"	"
"	"	5	82	"	"
"	"	6	94	"	"
"	"	7	103	"	"
"	"	9	119	"	"
"	"	10	119	"	"
"	"	11	128	"	"

1868.	No. of Dredging.	Fms.	Locality.
May 11	12	127	Off Sand Key.
" "	13	123	" "
" "	14	134	" "
" "	15	143	" "
" "	16	138	" "
" "	17	154	" "
" "	19	306	" "
" "	20	248	" "
" 15	1	100	" "
" "	3	100	" "
" "	4	100	" "
" "	5	100	" "
" "	6	100	" "
" 16	1	120	" "
" "	2 & 3	120	" "
" "	4 & 5	120	" "
" "	6	120	" "
1869.			
Jan. 15	1	6–7	S. of Tortugas.
" "	2	13	" "
" "	3	17	" "
" "	4	34	" "
" "	7	260	" "
" 16	1 & 2	30–32	W. of Tortugas.
" "	3	35	" "
" "	4	36	" "
" "	5	36	" "
" "	6	35	" "
" "	7	35	" "
" "	8	37	" "
" "	9	37	" "
" "	10	34	" "
" "	11	43	" "
" "	12 & 13	42	" "
" 17	1	43	" "
" "	3	124	" "
" "	5	502	" "
" 18	1	25	S. W. of Tortugas.
" "	3	60	" "
" "	4	115	" "
" "	5	214	" "
" "	6	306	" "
" "	7	389	" "
" "	8	468	" "

1869.		No. of Dredging.	Fms.	Locality.
Jan.	22	1	13	Between Rebecca Shoal and East Key.
"	"	2	11	S. of Rebecca Channel.
"	"	3	16½	" "
"	"	4	47	" "
"	"	5	118	" "
"	"	6	290	" "
"	"	7	349	" "
"	"	8	377	" "
"	"	9	416	" "
"	23	1	34	12 m. W. of Marquesas.
"	"	2	74	Off the Quicksands.
Feb.	10	1	42	Off Marquesas.
"	"	2	55	" "
"	"	3	40	" "
"	"	Several casts.	12–15	" "
"	11	1	107	S. of Marquesas.
"	"	2	132	" "
"	"	3	140	" "
"	"	5	296	" "
"	"	6	333	" "
"	15	1	105	Off Boca Grande.
"	"	2	122	" "
"	"	3	122	" "
"	"	4	125	" "
"	"	5	125	" "
"	"	6	90	" "
"	16	1	125	" "
"	"	3	327	" "
"	"	4	368	" "
"	"	5	405	" "
"	17	1	50	S. W. of Sand Key.
"	"	2	125	" "
"	"	3	138	" "
"	"	4	325	" "
"	"	5	87	" "
Mar.	4	1	450	Off Cojima, near Havana.
"	5	2	638	Off Cruz del Padre, Cuba.
"	10	1	315	Off Double-headed Shot Keys.
"	21	1	40	Off Conch Reef.
"	"	2	45	Off French Reef.
"	"	3	49	Off the Elbow Reef.
"	"	4	70	" "
"	"	5	60	Off Carysfort Reef.

1869.		No. of Dredging.	Fms.	Locality.
March	21	6	48	Off Carysfort Reef.
"	"	7	40	" "
"	"	8	35	" "
"	"	9	12	Off Turtle Harbor.
"	23	1	63	Off Carysfort Reef.
"	"	2	116	" "
"	"	3	138	" "
"	"	4 (Empty.)	293	" "
"	"	5	317	" "
"	"	6	320	" "
"	"	7	351	" "
"	31	1	52	" "
"	"	2	117	" "
"	"	3	206	" "
"	"	4	349	" "
April	1	2 & 3	9	Off Orange Key, Bahamas.
"	3	1	15	Off French Reef.
"	"	2	37	" "
"	"	3	44	" "
"	"	4		" "
"	"	5	75	" "
"	"	6	10	" "
"	21	1	135	Off Key West.
"	"	2	295	" "
"	"	3	140	" "
"	"	4	140	" "
"	"	5	120	" "
May	7	1	21	Off Tennessee Reef.
"	"	2	53	" "
"	"	3	85	" "
"	"	4	108	" "
"	"	5	114	" "
"	"	6	115	" "
"	"	7	124	" "
"	"	8	160	" "
"	"	9	174	" "
"	"	10	200	" "
"	8	2	41	Off Alligator Reef.
"	"	3	53	" "
"	"	4	68	" "
"	"	5	79	" "
"	"	6	88	" "
"	"	7	110	" "
"	"	8	110	" "

1869.		No. of Dredging.	Fms.	Locality.
May	8	9	113	Off Alligator Reef.
"	"	10	118	" "
"	"	11	138	" "
"	"	12	147	" "
"	"	13	156	" "
"	"	14	189	" "
"	"	15	238	" "
"	11	1	30	Off Conch Reef.
"	"	2	39	" "
"	"	3	49	" "
"	"	4	60	" "
"	"	5	77	" "
"	"	6	117	" "
"	"	7	139	" "
"	"	8	157	" "
"	"	9	169	" "
"	"	10	257	" "
"	13	1	30	Off Pacific Reef.
"	"	2	49	" "
"	"	3	60	" "
"	"	4	75	" "
"	"	5	98	" "
"	"	6	180	" "
"	"	7	233	" "
"	"	8	283	" "
"	"	9	287	" "

The following dredging stations were occupied by the U. S. Coast Survey steamer "Hassler," Lieut.-Commander P. R. Johnson, U. S. N., commanding, during her voyage from Boston to San Francisco, in 1871 and 1872. Prof. L. Agassiz was in charge of the scientific department; the dredgings were made by L. F. Pourtalès, Assist. U. S. Coast Survey.

1871.		No. of Dredging.	Fms.	Locality.
Dec.	29	1–4	75–100	Off Sandy Bay, Barbados.
"	30	5–8	17–100	" " "
1872.				
Jan.	18	9	15	Lat. 11° 49′ S., between the meridians
"	"	10	17	of 37° 10′ and 37° 27′ W., standing
"	"	11	40	off and on shore.
"	"	12	500	" " "
"	"	13	20	" " "
"	"	14	75	" " "
"	"	15	200	" " "

1872.		No. of Dredging.	Fms.	Locality.
Jan.	20	16	30	Off the Abrolhos, Brazil.
"	"	17	20	" " "
"	"	18	26	" " "
"	"	19	44	" " "
"	22	20	35	Off Cape Frio, Brazil.
"	"	21	45	" " "
Feb.	20	22	70	Lat. 32° 0′ S., Long. 50° 15′ W.
"	"	23	70	" " "
"	22	24	19	Lat. 34° 55′ S., Long. 54° 12′ W., off La Plata River.
"	29	25	7	Lat. 35° 12′ S., Long. 55° 30′ W., in La Plata River.
March	1	26	44	Lat. 37° 42′ S., Long. 56° 20′ W.
"	3	27	30	" 40° 22′ " 60° 35′
"	4	28	17	" 41° 17′ " 63° 0′
"	"	29	25	" 41° 15′ " 63° 50′
"	7	30	30	" 41° 40′ " 63° 13′
"	9	31	55	" 44° 52′ " 64° 10′
"	11	32	57	" 49° 40′ " 66° 50′
"	12	33	58	" 51° 26′ " 68° 5′
"	13	34	22	Off Cape Possession, Patagonia.
"	19	35	13	Anchorage at Sandy Point.
"	20	36		" at Port Famine.
"	21	37		" at Port Gallant.
"	27	38	135	Between Sholl Harbor and Cape Tamar, Straits of Magellan.
April	16	39	7–9	Talcahuano Bay.
"	25	40	35	Off Talcahuano Bay.
"	"	41	64	
"	"	42	66	
"	"	43	84	
"	27	44	2410	Lat. 35° 29′ S., Long. 75° 11′ W. Surface temp., 57.5°. Bottom temp., 35°. Dredge lost.
"	29	45	656	Two miles off Cumberland Bay, Juan Fernandez. Bottom temp., 39°.
"	"	46	1144	Three miles N. W. of Juan Fernandez. Bottom temperature, 36°.
May	2	47	65	Off Cumberland Bay.
"	"	48	220	" "
"	13	49	45	Off Valparaiso.

During the season of 1877 and 1878 the dredging operations from December to March were in charge of Alexander Agassiz, and the follow-

ing stations were occupied by the U. S. Coast Survey steamer "Blake," Lieut.-Comm. C. D. Sigsbee, U. S. N., commanding. The cruise extended from Key West to Havana, from Havana westward along the north coast of Cuba, from Key West to the Tortugas, thence to the northern extremity of the Yucatan Bank and Alacran Reef, to Cape Catoche and across to Cape San Antonio, returning to Key West, and from Key West to the Tortugas, and northward to the mouth of the Mississippi River.

1877–78.

Stat.	Fms.	Temperature. Surface.	Temperature. Bottom.	Locality.
1	801	73°	$39\frac{3}{4}$°	Off Morro Light.
2	805	77	$39\frac{3}{4}$	" "
3	924	$78\frac{1}{2}$	$39\frac{3}{4}$	" " (Bottom, soft coral ooze.)
4	936	$77\frac{1}{2}$	$39\frac{1}{2}$	" " " "
5	229 152	76	$49\frac{1}{2}$	Lat. 24° 15′ N., Long. 82° 13′ W. (Soft coral ooze.)
6	137			Lat. 24° 17′ 30″ N., Long. 82° 9′ W.
7 & 8	(Only mud brought up.)			
9	111	70	$55\frac{1}{2}$	Seven m. S. by W. from Sand Key.
10	37			Lat. 24° 44′ N., Long. 83° 26′ W.
11	37			Lat. 24° 43′ N., Long. 83° 25′ W.
12	36			Lat. 24° 34′ N., Long. 83° 16′ W.
13	742			Off Morro Light.
14	850 900			Lat. 23° 18′ N., Long. 82° 21′ W.
15	785			Near Morro Light, Lat. 23° 14′ N., Long. 82° 25′ W.
16	292	77	$55\frac{2}{3}$	Near Morro Light, Lat. 23° 11′ N., Long. 82° 23′ W.
17	320	76	$50\frac{1}{2}$	About 2 m. from Mariel, Lat. 23° 4′ N., Long. 82° 43′ W.
18	756	76	40	Off Mariel, Lat. 23° 7′ N., Long. 82° 43′ 30″ W.
19	310	76	$52\frac{1}{2}$	Off Bahia Honda, Cuba, Lat. 23° 3′ N., Long. 83° 10′ 30″ W.
20	220	76	62	Off Bahia Honda, Lat. 23° 2′ 30″ N., Long. 83° 11′ W.
21	287			" " Lat. 23° 2′ N., Long. 83° 13′ W.
22	100	77	71	" " Lat. 23° 1′ N., Long. 83° 14′ W.
23	190	77	64	" " Lat. 23° 1′ N., Long. 83° 14′ W.
24	342	78	50	" " Lat. 23° 2′ 30″ N., Long. 83° 13′ W.
25	635	78	$40\frac{1}{2}$	" " Lat. 23° 4′ N., Long. 83° 12′ 30″ W.
26	110	72	$58\frac{1}{2}$	Lat. 24° $37\frac{1}{2}$′ N., Long. 83° 36′ W.
27	392	73	$44\frac{1}{2}$	Lat. 24° 30′ N., Long. 83° 49′ W.
28	863	75	$39\frac{1}{2}$	Lat. 24° 34′ N., Long. 84° 0′ W.
29	955		$39\frac{1}{2}$	Lat. 24° 36′ N., Long. 84° 5′ W.
30	968		$39\frac{1}{2}$	Lat. 24° 33′ N., Long. 84° 34′ W.

Stat.	Fms.	Temperature. Surface.	Temperature. Bottom.	Locality.
31	1920		$39\frac{1}{2}°$	Lat. 24° 33′ N., Long. 84° 23′ W.
32	95			Lat. 23° 32′ N., Long. 88° 5′ W.
33	1568–1400	$72\frac{1}{2}°$	$40\frac{1}{2}$	Lat. 24° 1′ N., Long. 88° 58′ W.
34	600 400	81	(In 600 fms.) $40\frac{1}{2}$	Lat. 23° 52′ N., Long. 88° 56′ W.
35	804	78	$40\frac{1}{2}$	Lat. 23° 52′ N., Long. 88° 58′ W.
36	84	74	60	Lat. 23° 13′ N., Long. 89° 16′ W.
37	35			N. W. end of Alacran Reef.
38	20			Yucatan Bank, Lat. 23° 10′ N., Long. 88° 35′ W.
39	14			Sixteen miles N. of Jolbos Islands.
40	1323	77	40	Lat. 23° 26′ N., Long. 84° 2′ W.
41	860	73	$39\frac{1}{2}$	Lat. 23° 42′ N., Long. 83° 13′ W.
42	620		$39\frac{3}{4}$	Lat. 23° 53′ N., Long. 83° 4′ 30″ W.
43	339		45	Lat. 24° 8′ N., Long. 82° 51′ W.
44	539	$74\frac{1}{2}$	$39\frac{1}{2}$	Lat. 25° 33′ N., Long. 84° 35′ W.
45	101	75	$61\frac{3}{4}$	Lat. 25° 33′ N., Long. 84° 21′ W.
46	888		$39\frac{1}{2}$	Lat. 25° 43′ N., Long. 84° 47′ 30″ W.
47	321	$74\frac{1}{2}$	$46\frac{3}{4}$	Lat. 28° 42′ N., Long. 88° 40′ W.
48	533	66	$41\frac{3}{4}$	Lat. 28° 47′ 30″ N., Long. 88° 41′ 30″ W.
49	118			Lat. 28° 51′ 30″ N., Long. 89° 1′ 30″ W.

Stations 50 to 79 were occupied by Lieut.-Commander C. D. Sigsbee while in search of Pentacrinus.

Stat.	Fms.	Locality.		
50	119	Lat. 26° 31′ N., Long. 85° 53′ W.		
51	243–450	Off Havana, Lat. 22° 11′ N., Long. 82° 21′ W.		
52	158	"	"	Lat. 22° 9′ N., Long. 82° 23′ W.
53	242	"	"	
54	175	"	"	
55	242	"	"	Lat. 22° 9′ N., Long. 82° 21′ W.
56	175	"	"	Lat. 22° 9′ N., Long. 82° 21′ 30″ W.
57	177	"	"	Lat. 22° 9′ 15″ N., Long. 82° 21′ W.
58	242	"	"	Lat. 22° 9′ 30″ N., Long. 82° 11′ 30″ W.
59	158	"	"	
60	480	"	"	
61	243	"	"	Lat. 22° 9′ N., Long. 82° 1′. W.
62	80	"	"	
63	177	"	"	
64	122–240	"	"	
65	127	"	"	
66	80–100	"	"	

Stat.	Fms.	Locality.
67	128–240	Off Havana.
68	243–458	" "
69	100	" "
70	111	Off Sand Key.
71	458	Off Havana.
72	50	Off Sand Key.
73	220	Lat. 23° 25′ N., Long. 83° 11′ W.
74	287	Lat. 23° 25′ N., Long. 83° 11′ W.
75	292	Off Havana.
76	154	" "
77	240	" "
78	129	" "
79	175	" "

During the season of 1878–79 the dredging operations, from December to March, were in charge of Alexander Agassiz, and the following stations were occupied by the U. S. Coast Survey steamer "Blake," Commander J. R. Bartlett, U. S. N. The cruise extended from Key West to Havana, from Havana to Jamaica through the Old Bahama Channel and Windward Passage, from Jamaica to St. Thomas along the south coast of Hayti and Porto Rico. From St. Thomas the "Blake" visited Santa Cruz, Saba Bank, Montserrat, St. Kitts, Guadeloupe, Dominica, Martinique, St. Lucia, St. Vincent, the Grenadines, Grenada, and extended the dredgings south as far as the 100-fathom line off Trinidad, returned to St. Vincent, and finished the dredging operations at Barbados.

1878–79.

Stat.	Fms.	Temperature. Surface.	Temperature. Bottom.	Locality.	Nature of Bottom.
100	250 400			Off Morro Light.	
101	175 250			" "	
102	128½	78½°	69°	Caya Cruz to Lobos Light.	White coral sand.
103	438	79	49½	Old Bahama Channel.	
104	500	76½	45¾	" "	
105	452	77¾	48¾	" "	Wh. coral sand, gritty.
106	269½			" "	" " fine.
107	428			" "	
108	994	78	39	Off Nuevitas.	Sticky yellow gray ooze, very fine, & chalk rock.
109	1554	76	38¾	Off Cayo de Moa.	Soft gray glob. ooze.
110	1205	78	38¾	Off Cape Maysi.	Greenish black ooze.

Stat.	Fms.	Temperature. Surface.	Temperature. Bottom.	Locality.	Nature of Bottom.
111	1200	80°	39½°	Lat. 19° 7′ N., Long. 74° 52′ W.	Soft gray glob. ooze.
112	1050	82	39½	W. of Navassa Bank.	Brown mud.
113	634	82	43	Off E. end of Jamaica.	"
114	459			Lat. 17° 54′ N., Long. 76° 42′ W.	"
115	228			Lat. 17° 55′ N., Long. 76° 41′ 20″ W.	Dk. br. mud, very [soft.
116	150			Near former.	
117	874	82½	40	Lat. 17° 47′ 20″ N., Long. 67° 3′ 20″ W.	Gray gritty ooze.
118	238			Lat. 18° 12′ N., Long. 64° 55′ W., between St. Thomas and Santa Cruz.	
119	1105	80½	39	Same line.	Grayish glob. ooze.
120	1952	80¾	38¾	" "	Grayish br. glob. oz.
121	2393	80	39½	" "	Gray glob. ooze.
122	2412	77½	38¾	" " Lat. 17° 52′ 15″ N., Long. 64° 54′ 50″ W.	" "
123	1450	80½	38¾	Lat. 17° 49′ 15″ N, Long. 64° 53′ 30″ W.	Gray ooze and white coral sand, mixed.
124	580	81	42¾	Off Santa Cruz.	F. wh. cor. and sil. s.
125	300			Near Ham's Bluff.	
126	226	79	50½	Off Santa Cruz.	Gray sand.
127	38	80¾	76¾	" "	Sand, blk. sp. & br. shs.
128	180	81	60¼	Off Frederickstadt, Santa Cruz.	Gray ooze, sand.
129	314	85	48½	" " " "	Blue gray ooze, soft.
130	451	84	44½	" " " "	Soft gray ooze.
131	18	79	77	Off Santa Cruz, Ham's Bluff.	Coarse coral s. & shs.
	580	81	42¾		
132	115	77	65	" " Frederickstadt.	Rock and broken shs.
133	42			" "	Rock and broken shs.
134	248	81	54½	" " Frederickstadt.	Coarse s. and br. shs.
135	450	81	42½	" " "	Sand and gray ooze.
136	508	80	42½	" " "	Very soft gray ooze.
137	625	79½	41¼	" " "	Fine gray ooze.
138	2376	76½	38½	" "	Very fine light br. oz.
139	218	78½	51	" " Mt. Eagle.	Fine sand & coarse gr.
140	1097	80	38½	Off Virgin Gorda.	
141	861	78½	40¼	" "	Br. shells and oz.
142	27	78½	77¾	Flannegan Passage.	Sand and br. shells.
143	150	79	63¼	Off Saba Bank.	
144	21			On "	
145	270	79½	51	Off St. Kitts.	Fine sand, br. shells.
146	245	79½	52	" "	Very fine gr. sand, bl. spk., ooze.

Stat.	Fms.	Temperature. Surface.	Bottom.	Locality.	Nature of Bottom.
147	250	$79\frac{1}{2}°$	$52\frac{1}{2}°$	Off St. Kitts.	Fine sand, blk. spk.
148	208	$79\frac{1}{2}$	$55\frac{1}{4}$	" "	" "
149	60 150	79	76	" "	Fine ooze and lava spk.
150	$373\frac{1}{2}$	$81\frac{1}{2}$	45	Between St. Kitts and Nevis.	Ooze and coarse fragments of pumice.
151	356			Off Nevis.	
152	122	$79\frac{1}{2}$	$67\frac{1}{2}$	Off St. Kitts.	
153	303	$79\frac{1}{2}$	$48\frac{3}{4}$	Off Montserrat.	Lava sand, blk. spks., brk. sh.
154	298	80	$49\frac{1}{2}$	"	Lava sand.
155	88	80	69	"	
156	88	80	69	"	
157	120			"	
158	148			"	Stony bottom.
159	196	$80\frac{1}{2}$	$53\frac{3}{4}$	Off Guadeloupe.	Rocky bottom, lava chips.
160	393	$80\frac{1}{2}$	$43\frac{1}{2}$	"	Lava sand.
161	583	$81\frac{1}{2}$	41	"	"
162	734	81	40	"	
163	{ 769	80	$39\frac{3}{4}$	"	Fine lava sand.
	{ 878	"	"		Came up empty.
164	150	80		"	Hard bottom.
165	277	80	48	"	Fine soft gray ooze, bl. sp.
166	150	80	$59\frac{3}{4}$	"	
167	175	80	55	"	Sand bl. spks. & br. shs.
168				"	Lost the trawl.
169	101	$79\frac{1}{2}$	67	"	
170	309	80	$46\frac{1}{2}$	"	Lava sand and little oz.
171	183	80	$55\frac{1}{2}$	"	
172	{ 62 100 150 300	80	$74\frac{1}{2}$	"	
173	734	81	40	"	Fine grayish-br. ooze.
174	878	80	$39\frac{3}{4}$	"	
175	611	80	$40\frac{1}{2}$	Off Dominica.	Fine sticky br. ooze.
176	391	80	$43\frac{1}{2}$	"	D. br. o. & s., brk. shs.
177	118	80	65	"	Fine sand & brk. shs.
178	130	80	$61\frac{1}{2}$	"	Fine gr. s. with bl. sp.
179	824	$79\frac{1}{2}$	40	"	Fine br. ooze & sand.
180	982	$80\frac{1}{2}$	$39\frac{3}{4}$	"	Fine brown ooze.
181	118	80	65	"	
182	1131	81	$39\frac{1}{2}$	"	Fine br. ooze & sand.
183	252	79	$49\frac{1}{2}$	"	Fine soft d. br. m.
184	94	79	$69\frac{1}{2}$	"	Fine sand, dark brown mud.

Stat.	Fms.	Temperature. Surface.	Temperature. Bottom.	Locality.	Nature of Bottom.
185	333	80°	44°	Off Dominica.	Fine sand, dark brown mud.
186	98	80	66	" "	Fine sand, br. mud, and shells.
187	411	79½	43	" "	Fine sand, mud.
188	372	80	43	" "	Fine sand, black mud.
189	{84 / 120}	79	69½	" "	" "
190	542	79½	42	" "	Coarse sand, br. sh. fgts., bl. m.
191	{108 / 250}	79	64	" "	Fine sand, dark brown mud.
192	138	75	63¾	" "	
193	169	79½	51	Off Martinique.	Fine sand, dark mud, & shells.
194	442	80	41½	" "	Fine sand, ooze.
195	502½	80	41	" "	Fine gray sand, and ooze.
196	1030	80	39	" "	" " "
197	1224	80	39	" "	Light brown ooze.
198	136	79½	52½	" "	Rocky bottom.
199	136	79½	52½	" "	Rocky bottom.
200	472	80	41½	" "	Hard bot., very little dk. br. s.
201	565	80	40½	" "	Fine dark gray ooze.
202	210	78	48½	" "	Coarse sand and broken shells.
203	96	79	61	" "	Coarse sand and broken shells.
204	476		42½	" "	Fine sand, broken shells.
205	334	80	45½	" "	Fine sand and broken shells.
206	170	79	49	" "	Fine s., yell. gr., very sticky.
207	826	80	39¾	" "	Fine sand, brk. sh., ooze.
208	213	80	50½	" "	Hard bottom.
209	189	80	49¾	" "	" "
210	191			" "	Rough bottom.
211	357			" "	Fine yellow sand and brk. sh.
212	317	80	45½	" "	Fine gray sand and ooze.
213	357			" "	
214	892	79½	39½	" "	Sand and ooze.
215	226	79	51	Off St. Lucia.	Coarse sand and brk. shells.
216	154	79½	54½	" "	Hard bottom, fine sand.
217	398	80	43½	" "	
218	164	80	56	" "	
219	151	79	57	" "	
220	116	79	58½	" "	Rocky bottom.
221	423	80	42¾	" "	Gray ooze.
222	422	80	42½	" "	
223	146	79	56	Off St. Vincent.	Fine black sand.
224	114		57	" "	Rocky bottom, coral.
225	458	79½	41½	" "	Fine sand.

Stat.	Fms.	Temperature. Surface.	Bottom.	Locality.	Nature of Bottom.
226	424	79¼°	42½°	Off St. Vincent.	Fine sand, black sp., and ooze.
227	573	80	40½	" "	Fine sand, gray ooze.
228	785	81	39½	" "	Very fine gray sand and ooze.
229	1004	79¼	39½	" "	" " "
230	464	81	41½	" "	
231	95	80	61½	" "	Coarse sand and rock.
232	88	80	62	" "	
233	174	80	49½	" "	Rocky bottom.
234	306	80½	47	Off Bequia.	Very rough, fine gray sand.
235	1507	79	39	" "	Light brown ooze.
236	1591	79	39	" "	Fine gray ooze.
237	1290		38½	Off Grenadines.	V. f. sticky oz., brownish gray.
238	127	79½	56	" "	Fine coral sand.
239	338	80	45½	" "	Fine sand and ooze.
240	164	79¾	52¾	" "	Coral and broken shells.
241	163	80	53	" "	
242	842	80	39½	" "	Fine sand and gray ooze.
243	171	79½	51½	" "	
244	792	72	39	Off Grenada.	Gray ooze.
245	1058	77	39	" "	Sticky fine br. blue ooze.
246	154	79¾	56	" "	Fine gray ooze.
247	170	80	53½	" "	
248	161	80	53½	" "	Fine gray ooze.
249	262	80	47	" "	Coarse yellow sand.
250	421	80¼	41½	" "	Coral sand and ooze.
251	382	80¼	42	" "	Sand, little gray ooze.
252	306	80	44½	" "	Gray ooze.
253	92	79½	58½	" "	Coral and broken shells.
254	164	78½	57	" "	Coral and broken shells.
255	344	78	43½	" "	Dark gray ooze.
256	370	80	44½	" "	Fine sand and blue gr. ooze.
257	553	80	40½	" "	
258	159	80	53½	" "	
259	159	79½	53½	" "	
260	291	79½	47	" "	Fine gray ooze.
261	340			" "	
262	92	80	62	" "	Fine sand.
263	159	80	53½	" "	
264	416	80	42½	" "	Gray ooze.
265	576	79½	39¾	" "	
266	461	80½	41½	" "	
267	626	81	39½	" "	Light brown and gray ooze.
268	955	80	39½	" "	Gray ooze, rocky bottom.

Stat.	Fms.	Temperature. Surface.	Temperature. Bottom.	Locality.	Nature of Bottom.
269	124	80°	57½°	Off St. Vincent.	
270	75	80½	66	" "	
271	458	79½	41½	Off Bequia.	Fine sand.
272	76	79	64¾	Off Barbados.	Coarse sand & shells, hard bot.
273	103	79½	59½	" "	Coral & broken shells, yellow.
274	209	79½	53½	" "	Fine sand and ooze.
275	218	80	52½	" "	Fine sand, brown specks.
276	94	79½	61	" "	
277	106	80	58	" "	Hard rocky bottom.
278	69	78	68	" "	Coral bottom.
279	118	79¾	58½	" "	Very rough, very rocky bot.
280	221	80	50½	" "	Glob. sand.
281	288	80	46½	" "	Glob. ooze and broken shells.
282	154	81	56	" "	Coral sand and broken shells.
283	237	80	49	" "	Hard bottom.
284	347	80	44½	" "	Fine s. glob. ooze.
285	13–40			" "	
286	7–45			" "	
287	7½–50			" "	Coral sand and broken shells.
288	399	80	44½	" "	Hard bottom.
289	713	80½	40	" "	
290	73	80	70¾	" "	Coarse coral s., broken shells.
291	200	79¾	49¾	" "	Flat calc. stones.
292	56	80	74½	" "	Coral sand and broken shells.
293	82	80	64½	" "	" " "
294	137	80½	54½	" "	Hard bottom.
295	180	80	50¾	" "	" "
296	84	78	61½	" "	" "
297	123	80¼	56½	" "	Calc. stones.
298	120	80¼	61	" "	Broken shells and coral.
299	140	80½	56½	" "	Coral and corallines.
300	82	80½	60	" "	

No. 2.—*Ophiuridæ and Astrophytidæ of the Exploring Voyage of H. M. S. "Challenger," under* Prof. Sir Wyville Thomson, F. R. S. By Theodore Lyman.

PART II.

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This concluding portion of the Prodrome contains the Ophiuridæ not included in Part I., and the Astrophytidæ.

There are two new genera and sixty-three new species: *Amphiura*, 19 species; *Ophiocnida*, 2; *Amphilepis*, 3; *Ophiactis*, 7; *Ophiostigma*, 1; *Ophiopholis*, 1; *Ophiochondrus*, 1; *Ophioconis*, 2; *Ophiomyces*, 2; *Pectinura*, 2; *Ophiopeza*, 1; *Ophiothrix*, 4. Also of the genera already treated, there have since been detected in *Ophiochiton* and *Ophioglypha* one new species each; and in *Ophiacantha*, four. Of Astrophytidæ there are, *Astroclon*, 1 species; *Astrotoma*, 1; *Astroceras*, 1; *Astroschema*, 5; *Ophiocreas*, 4.

There is added an index of the species contained in the two parts, together with such other species from considerable depths as I have from time to time described; the whole forming a list of the greater portion of the deep-sea Ophiurans and Astrophytons now known.

Cambridge, Mass., December 25, 1879.

AMPHIURA.

TABLE OF SPECIES HEREIN DESCRIBED.

Two mouth-papillæ on each side. (*O. maxima* has a third rudimentary.)	Disk distinctly scaled on both sides.	Two tentacle-scales.		Disk-scales swollen, humpy, and irregular. Ten stout, sharp arm-spines. Basal mouth-papillæ wide and scale-like. Tentacle scales very large, one overlapping the other.	*maxima.*
			Radial shields narrow, about three times as long as wide.	Four straight, tapering arm-spines. Disk-scales delicate. Upper arm-plates thin.	*bellis.*
				Radial shields small and nearly or quite separated. Seven or eight short, blunt, crowded, thick arm-spines. Outer mouth-papilla scale-like.	*incana.*
		One tentacle-scale.		Radial shields very small, broad, about twice as long as wide. Five or six short, conical, barred spines. Upper arm-plates narrow and rounded.	*argentea.*
				Radial shields broad, about twice as long as wide. Three or four short, moderately stout arm-spines. Tentacle-scale minute. Radial shields and upper arm-plates wider than in *A. Stimpsoni.*	*acacia.*
			Radial shields narrow, about thrice as long as wide.	Six short, stout arm-spines. Disk-scales fine. Upper arm-plates narrow.	*constricta.*
				Four long, cylindrical arm-spines, the uppermost and lowest longest. Tentacle-scale large and rounded.	*iris.*
		No tentacle-scale.		Four tapering, equal arm-spines. Disk-scales rather large and spaced.	*tomentosa.*
	Disk below naked or with rudimentary scales.	Two (sometimes one) minute tentacle-scales. Radial shields long and narrow.		Five slender, tapering arm-spines. Under arm-plates squarish shield-shaped. Outer mouth-papilla spiniform. Upper arm-plates narrow.	*lanceolata.*
		One well-marked tentacle-scale.		Five tapering arm-spines. Mouth-shields wide.	*glabra.*
				Four or five tapering cylindrical arm-spines. Mouth-shields rounded.	*angularis.*
		No tentacle-scale.		Radial shield pear-seed shape. Four to five small, widely-spaced arm-spines.	*dilatata.*
Three mouth-papillæ on each side.				Two tentacle-scales. Four arm-spines. Inner mouth-papillæ thick; two outer small and sharp. Radial shields narrow and separated.	*concolor.*
Four mouth-papillæ on each side.		One tentacle-scale.	Three arm-spines, the middle one swelled.	Disk-scales fine; only central primary plate conspicuous. First under arm-plate small.	*dalea.*
				Disk-scales coarse; all primary plates conspicuous. First under arm-plate wide and large.	*cernua.*
				Four arm-spines. Disk naked below. Tentacle-scale minute and like a lip. Radial shields long and narrow, diverging inward.	*glauca.*
				No tentacle-scale. Four arm-spines. Disk scaled on both sides. Radial shields large, wide, and joined for half their length. Primary plates conspicuous.	*Verrilli.*
Five mouth-papillæ on each side.				Two tentacle-scales. Three middle mouth-papillæ longest. Point of mouth-angle occupied by lowest tooth.	*canescens.*
				One tentacle-scale. Mouth-papillæ squarish and crowded. Side mouth-shields large and wide. Disk-scales irregular, small, and crowded.	*patula.*

Amphiura maxima sp. nov.

Plate XI. Figs. 278-281.

Special Marks. — Disk covered on both sides with swollen, lumpy, irregular scales: ten stout, sharp arm-spines. Outer mouth-papillæ wide and scale-like. Two very large tentacle-scales, one overlapping the other.

Description of an Individual (Station 188). — Diameter of disk 15 mm. Length of arm about 135 mm. Width of arm, close to disk, without spines, 2.5 mm. One very large square mouth-papilla on each side of the angle, and a pair much smaller and more rounded at the apex; besides these, there may be distinguished a minute papilla outside the great flat one. Mouth-shields large, and much curved within, and prolonged by a rounded lobe without. Side mouth-shields very small, pear-seed shape, with the smaller end inward; they occupy the inner lateral sides of the mouth-shield, and are widely separated. Under arm-plates four-sided, broader than long, outer and inner edge slightly curved, and with feeble re-entering curves on the lateral sides. Side arm-plates short and high, scarcely prominent, meeting neither above nor below. Upper arm-plates small, little swollen, nearly round; but some distance out on the arm they are broader than long. Disk round, flat, and rather thick, having a notch over each arm; surface covered above and below with rather large, rounded, swollen, loosely overlapping scales, those in the interbrachial spaces being slightly larger. Radial shields pear-seed shape, little swollen, with a peak inward, separated their entire length by a row of three elongated scales, the inner one being surrounded by several much smaller. On the outer edge of the radial shields there is a row of small scales continuous with those on the margin of the disk. There are ten stout, pointed arm-spines, the two lowest being about twice as long as the others, much sharper, and usually curved. Two very large, flat tentacle-scales with curved edges, one on the inner margin of the tentacle-pore, which overlaps the one on the edge of the under arm-plate. Color in alcohol, straw.

Station 188, 28 fathoms, 2 specimens.

Amphiura bellis sp. nov.

Plate XI. Figs. 282-284.

Special Marks. — Disk covered above and below with delicate scales; two tentacle-scales. Radial shields narrow, about three times as long as wide; four straight tapering arm-spines; upper arm-plates thin.

Description of an Individual (Station 232). — Diameter of disk 7 mm. Arm long, slender, and tapering gradually; its width next the disk is 1 mm. One stout, short, blunt papilla on either side of the base of mouth-angle, and a pair, stout and bluntly pointed, at its apex. The tentacle-scales of the first pair are spiniform and rather conspicuous. Mouth-shields small and rounded, with sometimes a rounded angle within and a slight lobe without. Side mouth-shields three-sided, quite broad without, tapering within, where they do not meet. First under arm-plate six-sided and rather larger than usual; those

beyond squarish, about as long as broad, with outer side nearly straight, lateral sides a little re-enteringly curved, and usually a very short truncated angle within. Side arm-plates small, and not strongly projecting, meeting neither above nor below. Upper arm-plates thin, of a pretty regular transverse oval shape, with lateral corners well rounded Disk rather thick, and slightly lobed, covered above and below with small, rather thin, overlapping scales, among which the primaries are scarcely to be distinguished ; those near the margin and underneath are finest, being 9 or 10 in 1 mm. long. Radial shields long, narrow and pointed within ; length to breadth 2 : .7 ; they are separated their whole length by a narrow wedge composed of scales longer than those of the neighboring disk. Four moderately stout, cylindrical, tapering arm-spines, of equal lengths, and somewhat longer than the arm-joints. Two minute rounded tentacle-scales, one on the side arm-plate, the other on the under arm-plate. Color in alcohol, very pale brown.

The young of this species has sometimes only one tentacle-scale.

Station 232, 345 fathoms, 9 specimens. Station 174 (var. ?), 210 – 610 fathoms, 1 specimen.

Amphiura incana sp. nov.

Plate XI. Figs. 285 - 287.

Special Marks. — Disk scaled on both sides. Two tentacle-scales. Radial shields narrow, about three times as long as wide, nearly or quite separated. Lower scaling coarse. Seven or eight short, blunt, crowded, very thick arm-spines.

Description of an Individual (Station, Simon's Bay, Cape of Good Hope). — Diameter of disk 7 mm. Arms about 70 mm. long, and slender ; close to disk their width without spines is 1.3 mm. One short wide curved papilla each side of mouth-angle, and a pair, stout and bluntly pointed, at the apex of the mouth-angle above ; the tentacle-scales of the first pair are conspicuous. Mouth-shields small, of a wide diamond-shape, with outer angle truncated. Side mouth-shields much longer than wide, tapering slightly within, where they nearly or quite meet ; outer ends much rounded. Under arm-plates nearly square, with rounded corners, and outer edge a little re-enteringly curved. Side arm-plates rather thick but not prominent, meeting neither above nor below. Upper arm-plates small, narrow, squarish with rounded corners ; narrow within, broader without. Disk round, not very thick, covered with thin, very small overlapping scales : on the upper surface there are 5 or 6 in the length of 1 mm. Radial shields small, of a long pear-seed shape, with outer edge rounded, separated their entire length by a wedge of three rows of crowded, closely overlapping scales. Just outside the radial shields there are numerous fine scales. On the under surface of disk the scaling is much finer, there being about 12 in the length of a mm. Eight very short, stout, broad, nearly equal flattened arm-spines ; the two upper spines are somewhat broader than the others. Two minute rounded tentacle-scales on the side arm-plate. Color in alcohol, pale straw.

Station, Simon's Bay, Cape of Good Hope, 10 – 20 fathoms, 12+ specimens.

Amphiura argentea sp. nov.

Plate XI. Figs. 288-290.

Special Marks. — Disk scaled on both sides. One tentacle-scale. Radial shields very small; about twice as long as broad. Five or six short, conical arm-spines. Upper arm-plates narrow and rounded.

Description of an Individual (Station 171). — Diameter of disk 4 mm. Length of arm about 22 mm. Width of arm near disk 1 mm. One rather long, flat papilla on either side of the base of the small, short mouth-angle, and a pair, much rounded, at apex. Scales of first pair of mouth-tentacles long and rather conspicuous. Mouth-shields much wider than long, rounded, with a wide curve within, and outer side feebly curved. Side mouth-shields very narrow within, where they meet; wider without. First under arm-plate small and narrow, being squeezed between the outer ends of the side mouth-shields; those beyond are as broad as long, bounded without by a clean curve, on lateral sides by slightly re-entering curves, and within by a truncated angle. Side arm-plates very short, so that there is a considerable naked space between them on the sides of the arm; they stand well out, forming a strong spine-ridge. Upper arm-plates narrow, longer than broad, nearly pentagonal, with rounded corners and an angle inward. Disk delicate, covered above and below with minute, thin, nearly uniform, overlapping scales; 9 or 10 in the length of 1 mm. where they are smallest. Radial shields very small, slightly sunken, of a pear-seed shape, nearly or quite touching without, separated within by a narrow wedge of minute scales; length to breadth .9 : .3. Five or six short, nearly equal, stout arm-spines, whereof the lower are cylindrical and tapering, and the upper somewhat flattened and wider; lengths to that of an under arm-plate, .6, .6, .5, .6, .6, .7, .4. Near tip of arm there are three long, sharp, and very slender spines, twice as long as the arm-joints: this so great variation of form is rare in *Amphiura*. One oval tentacle-scale. Color in alcohol, nearly white.

Station 171, 600 fathoms, 1 specimen.

Amphiura acacia sp. nov.

Plate XI. Figs. 292-294.

Special Marks. — Disk scaled on both sides. One minute tentacle-scale. Three short, moderately stout arm-spines. Radial shields short and wide.

Description of an Individual (Station 235). — Diameter of disk 4.5 mm. Length of arm, about 32 mm. Width of arm near disk, 1 mm. One flat rounded papilla on each side of the mouth-angle, and a pair, blunt and thicker, at the apex. Scales of the first pair of mouth-tentacles flat, and low down, so as to seem nearly on a level with the outer mouth-papilla. Mouth-shields small, rounded, longer than broad, widest without, and having a rounded point inward. Side mouth-shields three-sided, short and broad, widely separated within. Under arm-plates narrow, longer than broad, five-sided, with an angle within, outer side

nearly straight, and lateral sides a little re-enteringly curved. Side arm-plates somewhat flaring, with a well-marked spine-ridge, meeting narrowly above and barely separated below. Upper arm-plates twice as broad as long, with a clean curve within and the outer side nearly straight, but having usually a feeble lobe in the centre. Disk rather thick, covered with fine, curved, rather thin, overlapping scales, which are largest in the centre, where may be distinguished an ill-marked rosette of primary plates; those near the margin are much finer (about 8 in the length of 1 mm.): on the lower surface they become thinner and near the mouth-shield are hard to distinguish. Radial shields short and wide, curved on the interbrachial side, straight on the brachial; barely touching without, separated within by a narrow wedge of four or five scales; length to breadth, 1.1 : .6. Three short, cylindrical, gently tapering, blunt, equal arm-spines about .5 mm. long. One minute, rounded tentacle-scale. Color in alcohol, pale gray.

Station 235, 565 fathoms, 3 specimens.

Amphiura constricta sp. nov.

Plate XI. Figs. 295-298.

Special Marks. — Disk finely scaled on both sides. One tentacle-scale. Radial shield narrow, about thrice as long as wide. Six short, stout arm-spines. Upper arm-plates narrow.

Description of an Individual (Station, Port Jackson).—Diameter of disk, 5 mm. Length of arm, 30 mm. Width of arm near disk, 1 mm. One minute, rounded papilla at base of mouth-angle, on either side, and a pair, much larger, at the apex. Above may be seen the small scales of first mouth-tentacles, which resemble the outer mouth-papillæ. Mouth-shields wider than long, of a three-sided or wide heart-shape with rounded angles. Side mouth-shields long and narrow, especially within, where they do not meet. First under arm-plate small and very narrow; those beyond are small and narrow, a little longer than wide, and four-sided with rounded corners; they cover only a small portion of the under side of the arm. Side arm-plates small and not projecting. Upper arm-plates small and covering only a portion of the upper side; pretty regular transverse oval, about twice as broad as long. Disk thick and somewhat puffed, covered with regular, small, rounded, overlapping scales, which are somewhat larger near the centre, where small round primary plates, widely separated by smaller scales, may be distinguished; below and near margin of disk, the scaling is finer and more delicate, about 10 in the length of 1 mm. Radial shields long, narrow, and slightly curved, acute within, separated their whole length by a wedge of many irregular scales of several sizes; length to breadth, 1 : .3. Six small, short, stout, blunt, peg-like, equal arm-spines about .3 mm. long, of which one or two are microscopically rough at their ends. The spines at tip of arm are similar, but proportionately longer. One rather large oval tentacle-scale.

Station, Port Jackson, Australia, 2 to 10 fathoms, 1 specimen.

Amphiura iris sp. nov.

Plate XI. Figs. 302-304.

Special Marks. — Disk scaled above and below; one large oval tentacle-scale: four long arm-spines, the uppermost and lowest longest.

Description of an Individual (Station 236). — Diameter of disk 5 mm. Width of arm without spines 1.2 mm. One short, stout, somewhat flattened blunt papilla on each side of the mouth-angle, and a pair, similar, but somewhat smaller, at its apex. The large and broad scales of the first pair of tentacles are low down and conspicuous. Mouth-shields of a very wide heart-shape, much wider than long, with a rounded angle within. Side mouth-shields thick, long triangular, tapering inward where they do not meet. First under arm-plate usually large; of a diamond shape, with its angles more or less truncated; the plates beyond are longer than wide, with outer side curved and widest, lateral sides re-enteringly curved and a truncated angle within. Side arm-plates stout and rather prominent, meeting neither above nor below. Upper arm-plates fan-shaped, with inner angle more or less rounded, or truncated, and outer side gently curved. Disk covered above and below, with moderately coarse, crowded, irregular scales, those of the interbrachial spaces being more elongated, and those on the under surface somewhat obscured by skin. Toward the centre of the disk there are 7 or 8 scales in the length of 1 mm. Radial shields much longer than wide, slightly curved, somewhat swollen, tapering at both ends and widest in the middle; separated their whole length by a row of three or four large scales; length to breadth, 2 : .6. Four long, cylindrical, tapering arm-spines, whereof the uppermost and lowest are longest, and equal to $1\frac{2}{3}$ arm-joints. One large tentacle-scale. Color in alcohol, pale gray.

Station 236, 420–775 fathoms, 1 specimen.

Amphiura tomentosa sp. nov.

Plate XI. Figs. 299-301.

Special Marks. — Disk scaled on both sides with rather large, spaced scales; those below somewhat obscured by thick skin; four tapering, equal arm-spines; no tentacle-scale.

Description of an Individual (Station, Balfour Bay, Kerguelen Isl.). — Diameter of disk 6.5 mm. Width of arm close to disk, without spines, 1 mm. One very small short mouth-papilla, often obscured by skin, on each side of the mouth-angle, and a pair, larger and rounded, at the apex. Mouth-shields irregular, small, rounded triangular, with a small peak inward. Side mouth-shields longer than broad, wider without than within, where they just meet; both they and the mouth-shields are somewhat obscured by skin. Under arm-plates narrow, longer than broad, pentagonal, with a blunt angle inward, small re-entering curves on the lateral sides, and outer lateral corners rounded. Side

arm-plates moderately projecting, nearly meeting above and below. Upper arm-plates somewhat broader than long, transverse oval, with a deep curve within, and a gentler one without. Disk thick and round, covered with thin, rather large, rounded scales, which are seldom overlapping, and often separated from each other by much smaller ones. Radial shields small, quite narrow, much wider without than within, where they form a sharp angle, widely separated by a wedge of three or four scales. The interbrachial space on the under surface is covered by fine scaling, which is often quite obscured near the mouth-shields by skin. Four equal, rather long, stout, and bluntly pointed arm-spines. Large round tentacle-pores, but no scales. Color in alcohol, pale gray.

Station, Balfour Bay, Kerguelen Isl., 20–60 fathoms, 1 specimen.

Amphiura lanceolata sp. nov.

Plate XI. Figs. 305-307.

Special Marks. — Disk nearly or quite naked below. Two small tentacle-scales. Radial shields long and narrow. Five slender, tapering arm-spines. Upper arm-plates narrow. Under arm-plates squarish shield-shaped. Outer mouth-papilla spiniform.

Description of an Individual (Station 169). — Diameter of disk 4 mm. Arms long and slender, about .7 mm. wide at the base. One slender, sharply pointed mouth-papilla on each side of the mouth-angle, and a pair, short and much rounded, at the apex. Mouth-shield small, thick, nearly oval. Side mouth-shields three-sided, large and thick, as broad as long, curving round the inner angles of the mouth-shield, but not meeting within. Under arm-plates narrow, longer than wide, pentagonal in shape, with an obtuse, or truncated angle inward, outer edge nearly straight, and re-entering curves on the lateral sides. Side arm-plates not prominent, nearly meeting above and below. Upper arm-plates much rounded triangular, with angle inward. Disk flat, with deep constrictions in the interbrachial spaces. The scaling of upper surface of disk is rounded and overlapping, and is much coarser in the centre, where also the six primary plates may be distinguished: near the margin there are from 8 to 10 scales in the length of 1 mm. Radial shields long and narrow, sharply pointed within; joined without, where the ends are much rounded, and separated within by a wedge of five or six scales. Interbrachial space on the under surface naked, or with scattered, scarcely discernible scales. Five rather long, slender, cylindrical, tapering, equal arm-spines about .6 mm. long. Two small rounded tentacle-scales, one on the under arm-plate, and one on the side arm-plate. Color in alcohol, pale gray.

Station 169, 700 fathoms, 1 specimen.

Amphiura glabra sp. nov.

Plate XI. Figs. 308 - 310.

Special Marks. — Disk below naked. Mouth-shields wider than long. Five stout, tapering arm-spines. One tentacle-scale.

Description of an Individual (Station 214). — Diameter of disk 5 mm. Length of arm about 20 mm. Width of arm close to disk, without spines, .8 mm. One stout mouth-papilla in shape of an elongated cone on each side, and a pair, thick and rounded, at the apex of the mouth-angle. Mouth-shields broader than long, rudely triangular, with outer edges much rounded, and a small peak within. Side mouth-shields small, longer than broad, wide without, tapering inward, where they do not quite meet. Under arm-plates narrow, longer than broad, squarish, with re-entering curves on the lateral sides, outer corners rounded, and often an obtuse truncated angle within. Side arm-plates of moderate size, and slightly flaring, meeting neither above nor below. Upper arm-plates somewhat arched, rudely triangular, with outer edge rounded, and a blunt angle within; further out on the arm they become transverse oval. Disk flat and lobed, covered above with thin, rather indistinct scales; those in the centre coarser and more rounded; those in the interbrachial spaces narrower and more closely overlapping. Radial shields short pear-seed shape, longer than broad, separated their entire length by a narrow wedge-row of small scales. Interbrachial spaces on the under surface naked. Five rather stout, tapering arm-spines, somewhat longer than the arm-joints, placed close together on the side arm-plate. One rather large round tentacle-scale near the inner angle of the under arm-plate. Color in alcohol, nearly white.

Station 214, 500 fathoms, 1 specimen.

This species is allied to *A angularis*, but has a finer build; side arm-plates less prominent; side mouth-shields smaller, and radial shields shorter and wider.

Amphiura angularis, sp. nov.

Plate XI. Figs. 311 - 313.

Special Marks. — Disk below naked, or with a few rudimentary scales. One well-marked tentacle-scale. Four or five tapering cylindrical arm-spines. Mouth-shields rounded.

Description of an Individual (Station 150). — Diameter of disk 9 mm. Length of arm 45 mm. Width of arm, without spines, close to disk, 1.2 mm. One long, tapering, pointed mouth-papilla on each side, and a pair, short, blunt, and much rounded, at the apex of the mouth-angle. The tentacle-scale of the first pair is large and spiniform. Mouth-shields rather large, nearly circular, with a small peak within. Side mouth-shields large, three-sided, broad without, and curving downward about the mouth-shield, narrow and separated within. First under arm-plate very small and squarish; those beyond are nearly square

and rather narrow, with outer corners rounded, and slight re-entering curves on the lateral sides. Side arm-plates wide, prominent, and much swollen along the spine-crest ; separated below, nearly or quite meeting above. Upper arm-plates transverse oval, much wider than long, with well-rounded lateral ends. Disk flat and angular, covered above with coarse, rounded, overlapping scales, the five primaries being but little larger than the other scales ; the scaling on the interbrachial spaces is finer than in the central portion. Radial shields much longer than broad, tapering towards each extremity, with the inner point acute, separated their entire length by two or three rows of irregular scales ; length to breadth 2 : .7. The scales of the margin continue round the outer end of the radial shields. Interbrachial space below only about one third covered with minute scaling; the rest of the space is naked. Four stout, blunt, tapering, cylindrical arm-spines, evenly spaced on the side arm-plate. One stout, round tentacle-scale on the inner side of the tentacle-pore. Color in alcohol, disk gray, arms straw.

Station 150, 150 fathoms, 12+ specimens.

Amphiura dilatata sp. nov.

Plate XI. Figs. 314-316.

Special Marks. — Disk naked below. Radial shields narrow pear-seed shape. Four or five small, widely spaced arm-spines. No tentacle-scales.

Description of an Individual (Station 141). — Diameter of disk 5 mm. Length of arm 23 mm. Width of same without spines, close to the disk, .7 mm. At the base of the mouth-angle, on each side, is a long, very slender mouth-papilla, and a pair, blunt and rounded, at the apex. Mouth-shield small, short diamond-shape, with much rounded angles. Side mouth-shields small and curved, narrow within, where they nearly or quite meet ; outer end wide club-shaped. Under arm-plates narrow, longer than broad, squarish, with re-entering curves on the lateral sides, and the outer edge nearly straight. Side arm-plates very small, not prominent, nearly or quite meeting above, separated below. Upper arm-plates transverse oval, with the inner curve stronger than the outer, and the lateral corners pointed ; there is a slight longitudinal ridge. Disk rather thick and slightly puffed ; primary plates widely separated and scarcely to be distinguished from the general scaling, which is fine, regular, and overlapping, having about 10 scales in the length of 1 mm.; those of the interbrachial spaces are smallest and most closely overlapping. Radial shields small, and slightly swollen, narrow pear-seed shaped, separated their entire length by a narrow wedge-row of scales ; a pair of short, stout scales at their outer ends. Under surface of disk naked. Five short, tapering, blunt arm-spines, evenly spaced on the side arm-plate, and standing at right angles to the arm ; the middle spine is stoutest. Large tentacle-pores, but no tentacle-scales. Color in alcohol, disk gray, arms straw.

Station 141, 98 fathoms, 12+ specimens.

Amphiura concolor sp. nov.

Plate XII. Figs. 317 - 319.

Special Marks. — Three mouth-papillæ on each side, the inner one large and thick, the two outer small and bead-like. Two, sometimes only one, small tentacle-scales. Four arm-spines. Radial shields narrow and separated.

Description of an Individual (Station 195). — Diameter of disk 8 mm. Length of arm 65 mm. Width of arm close to disk, without spines, 1 mm. Two very short, small mouth-papillæ each side of the mouth-angle, and a pair, large, rounded, much swollen at its apex. Four large, thick teeth, with a square cutting edge. Mouth-shield wide spear-head shaped, with a blunt angle within, and the inner sides slightly curved. Side mouth-shields large, broad without, tapering inward, where they just meet. Basal under arm-plates large, pentagonal with the inner angle truncated, broader than long, outer edge straight, lateral sides re-enteringly curved. Side arm-plates rather small, projecting moderately, meeting neither above nor below. Upper arm-plates short and wide, of a transverse pointed oval form, with outer and inner edge slightly curved. Disk round and flat, but rather thick, covered with irregular, overlapping scales; those in the interbrachial spaces being somewhat coarser than the others. Radial shields long and narrow, with outer end rounded, and an acute angle inward, separated their entire length by a single row of scales. Interbrachial spaces on the under surface covered by similar, but finer, scaling. Four short, blunt, rather slender arm-spines, the upper one being slightly shortest. Two small, rounded tentacle-scales, one on the brachial side of the tentacle-pore and one on the side arm-plate. On some pores there is but a single scale. Color in alcohol, straw.

Station 195, 1425 fathoms, 2 specimens. Station 191, 800 fathoms, 12+ specimens.

Amphiura dalea sp. nov.

Plate XII. Figs. 320 - 322.

Special Marks. — Four mouth-papillæ on a side. Three arm-spines, the middle one swollen. One tentacle-scale. Disk-scales fine, only the central primary plate being conspicuous. First under arm-plate small.

Description of an Individual (Station 325). — Diameter of disk 9 mm. Width of arm close to disk, without spines, 1.3 mm. Three stout, close-set papillæ on either side of the mouth-angle, and two large and much rounded at the apex; of those on the sides the outermost is largest. Mouth-shields small, triangular, a little longer than wide, rounded on all sides except within, where is a point. Side mouth-shields large, broad without, tapering inward where they just meet. First under arm-plate very small; those beyond are broader than long, angular, and with re-entering curves on the sides where are the tentacle-pores; still farther out they are triangular, with outer edge much curved,

and a truncated angle within. Side arm-plates short, not much projecting, meeting above beyond the first upper arm-plate, and below beyond the seventh or eighth. Upper arm-plates slightly swollen, very short and wide, of a transverse oval shape, and with a small longitudinal ridge. Disk flat and tolerably thick, covered with thin, small, flat, overlapping scales, with one somewhat larger rounded primary in the centre ; about 4 scales in the length of 1 mm. Radial shields long and broad, bluntly pointed within, nearly or quite separated their entire length by a narrow wedge of scales. On the interbrachial spaces on the under surface the scaling is much finer than that above, there being about 15 in the length of 1 mm. Three tapering, rather sharp arm-spines, the upper one being shorter than the other two, and the middle one much the stoutest, and swollen. One small longer than broad tentacle-scale on the brachial side of the tentacle-pore ; a little way out on the arm there usually is no tentacle-scale. Color in alcohol, pale straw.

Station 325, 2,650 fathoms, 5 specimens.

Amphiura cernua sp. nov.

Plate XII. Figs. 323-325.

Special Marks. — Four mouth-papillæ on each side. One tentacle-scale. Three arm-spines, the middle one swelled. Disk-scales coarse ; all primary plates conspicuous. First under arm-plate wide and large.

Description of an Individual (Station 241). — Diameter of disk 5.7 mm. Length of arm about 24 mm. Width close to disk, without spines .7 mm. Four mouth-papillæ on each side, of which three are short and blunt (the inner one being more pointed), and two at the apex of the mouth-angle are larger and more swollen. Mouth-shields small, flat, triangular, with a blunt angle inward and outer edge curved. Side mouth-shields broad without, and tapering inward, where they just meet. Under arm-plates large, with a long angle within and slight re-entering curves on the lateral sides. Side arm-plates slightly swollen, meeting below some distance out on the arm, and above beyond the first upper arm-plate. Upper arm-plates transverse oval, slightly swollen, with outer and inner edges much curved. Disk flat and slightly angular, covered with thin, semicircular, overlapping scales, the six primary plates being much the largest ; the scaling in the interbrachial spaces is somewhat coarser than on the rest of the disk. Radial shields very large and broad, somewhat longer than wide, of a blunt pear-seed shape ; joined without, separated within by a wedge of two small scales. On the under surface the interbrachial space is covered with very minute scaling. One large tentacle-scale longer than broad. Three short arm-spines, the upper one longest and slender, while the middle one is strongly swollen at its base. Color in alcohol, straw.

Station 241, 2,300 fathoms, 1 specimen.

Amphiura glauca sp. nov.

Plate XII. Figs. 326 - 328.

Special Marks. — Four mouth-papillæ on each side. One tentacle-scale minute and like a lip. Four slender arm-spines. Radial shields long and narrow, and diverging inward. Disk naked below.

Description of an Individual (Station 232). — Diameter of disk 5.5 mm. Width of arm close to disk 1 mm. Four short pointed mouth papillæ on each side of the mouth-angle, of which that at the apex is much the largest and most rounded. Mouth-shield small, with a rounded angle inward, and outer edge curved. Side mouth-shields small, long triangular, somewhat curved, just meeting within. Under arm-plates small, longer than wide, with re-entering curves on the lateral sides, outer corners rounded and a truncated angle within. Side arm-plates small and little projecting, meeting neither above nor below, till some distance out on the arm. Upper arm-plates small, a little broader than long, bounded within by a deep curve, and without by a gentler one, having a small ridge in the centre, which forms a continuous line along the arm. Disk rather thick, naked below, but covered above with very minute rounded scales, about 7 in the length of 1 mm. where they are finest. Radial shields long and very narrow, tapering inward to a blunt point; they are joined without, and separated within by several small scales. Four slender tapering arm-spines, the upper and under being usually somewhat longer than the two in the middle. One very small lip-like tentacle-scale, on the inner side of the tentacle-pore. Color in alcohol, dull gray.

Station 232, 340 fathoms, 4 specimens. Station 236, 420 fathoms, 1 specimen.

Amphiura Verrilli sp. nov.

Plate XII. Figs. 329 - 331.

Special Marks. — Four mouth-papillæ on each side. Four arm-spines. No tentacle-scales. Radial shields large and wide, and joined for half their length.

Description of an Individual (Station 54). — Diameter of disk 6 mm. Width of arm, without spines, close to disk, 1 mm. Four short, blunt mouth-papillæ on each side, the two at the apex being largest and conical; between them may be seen the lowest tooth, having a broken edge. Mouth-shields small, rounded, with a slight angle within. Side mouth-shields large, narrow within, where they meet; broader without, where they curve partially round the mouth-shield. First under arm-plate very small; those beyond are swollen, narrow, longer than broad, having the outer edge much rounded, deep re-entering curves on the lateral sides, and a short, straight side within. Side arm-plates small, separated below, but just meeting above. Upper arm-plates much broader than long, transverse oval, with the inner edge nearly straight, outer edge curved, and blunt angles on the lateral sides. Disk flat, moderately thick and

slightly angular, covered with small, thin, irregular, overlapping scales; there are six large, widely separated primary plates, one round one in the centre, surrounded by five others broader than long. Radial shields large, longer than wide, of an elongated pear-seed shape, their pointed inner ends being separated by two small, angular scales. Interbrachial space on the under surface covered by fine overlapping scales, smaller than those above. Four arm-spines standing close together on the side arm-plates; they are about as long as an arm-joint, and rather slender and tapering, except the one next the lowest, which is strongly swollen at the base. Large round pores, but no tentacle-scales. Color, gray.

Station 54, 2650 fathoms, 1 specimen.

Amphiura canescens sp. nov.

Plate XII. Figs. 332-334.

Special Marks. — Five mouth-papillæ on each side, of which the three middle ones are longest; point of mouth-angle occupied by the lowest tooth. Two tentacle-scales. Three arm-spines about as long as a joint.

Description of an Individual (Station 171). — Diameter of disk 5 mm. Arms long and slender. Width of arm, close to disk, without spines, 1 mm. Five stout, blunt mouth-papillæ on either side of the mouth-angle, the three middle ones being longer, broader, and more flattened than the rest. One large, triangular papilla, or tooth, at apex of jaw. Mouth-shields broad triangular, with blunt angles and outer edge much rounded. Side mouth-shields long and narrow, but slightly swollen, broader without than within, where they just meet. First under arm-plate small, pentagonal, with an angle inward and slight re-entering curves on the lateral sides; the other basal plates are large, with outer edge curved, and wider than the inner, and with lateral sides re-enteringly curved. Side arm-plates not prominent, meeting neither above nor below at the base of the arm. Upper arm-plates broader than long, transverse oval, with lateral ends slightly pointed. Disk flat, but rather thick, its upper surface covered with small, slightly swollen, irregularly shaped, overlapping scales, about 5 in the length of 1 mm. where they are coarsest. Radial shields blunt pear-seed shaped, slightly pointed within, separated by one large and several small scales. Interbrachial spaces on the under surface covered by the same kind of scaling. Three stout, tapering, bluntly pointed arm-spines, about as long as a joint, the lowest slightly longer than the others, placed close together on the side arm-plate. Two tentacle-scales, the one on the brachial side small and narrow, the interbrachial one much larger, with wide, rounded edge. Color in alcohol, nearly white.

Station 171, 600 fathoms, 2 specimens.

Amphiura patula sp. nov.

Plate XII. Figs. 335-337.

Special Marks. — Five (sometimes only four) mouth-papillæ on each side. One tentacle-scale. Mouth-papillæ squarish and crowded. Side mouth-shields large and wide. Disk-scales small, irregular, and crowded.

Description of an Individual (Station 156). — Diameter of disk 14.5 mm. Width of arm close to disk, without spines, 2 mm. Five (sometimes only four) squarish, crowded mouth-papillæ on either side, whereof the outermost and innermost are largest; besides these there is an odd one at the centre of the apex. Mouth-shields small, rounded triangular, with a blunt angle inward. Side mouth-shields short and stout, rudely triangular in shape, the inner angles not quite meeting at the apex of the mouth-shield. Under arm-plates pentagonal, with inner angle sometimes truncated, outer edge slightly rounded, and small re-entering curves on the lateral sides. Side arm-plates narrow, bent, not very prominent, meeting above, but just separated below. Upper arm-plates much broader than long, transverse oval, with outer and inner edges gently curved. Disk flat, covered with thin, flat, irregular, crowded scales, among which six small widely separated primary plates are with difficulty distinguishable. Radial shields large and broad, of a wide pear-seed shape, separated their entire length by a narrow wedge of three or four scales. On the under surface the scales are much finer and more rounded. Three short, round, bluntly tapering arm-spines, the middle one larger than the others but not so long as an arm-joint, and all placed low on the side arm-plate. Only one longer than wide, somewhat swollen tentacle-scale, on the brachial side of the tentacle-pore. Except that it has usually five, instead of four, mouth-papillæ on a side, this species stands related to *A. dalea*, from which it is distinguished by smaller arm-spines, different under arm-plates, and coarser, more irregular scaling. Color in alcohol, grayish.

Station 156, 1975 fathoms, 4 specimens.

Note. — The following are species previously known and now brought back by the "Challenger," namely, *A. squamata*, *A capensis*, *A Otteri*, *A. duplicata*, *A. Studeri* (*A. antarctica*), *A. depressa*.

Amphiura capensis Ljn.

Amphiura capensis. Öf. Kong. Akad. Oph. Viv., 1866, p. 320.

Station 141, Lee's Point, Cape Town, 98 fathoms, 12+ specimens.

Amphiura duplicata Lym.

Amphiura duplicata. Ill. Catal., No. VIII., Pt. 2, p. 19.

Station 56, 1,075 fathoms, 4 specimens.

Quite common in less depths throughout the West Indies. *A. duplicata* is

somewhat variable; and, especially, the first under arm-plate is not always broken in two. Numerous specimens from the second "Blake" Expedition show usually only three arm-spines; three and often four irregular mouth-papillæ on each side, and disk-scales varying in thickness.

Amphiura squamata Sars.

Amphiura squamata. Middelhav. Lit. Fauna, II., 1857, p. 84.

Station 141, 98 fathoms. Station 163, 120 fathoms, 1 specimen.

Such diverse localities further prove the cosmopolite nature of this species.

Amphiura Otteri? Ljn.

Amphiura Otteri? Öf. Kong. Akad. Dr. Goës, Oph., 1871, p. 631.

Station 76, 900 fathoms, 2 specimens. Station 45, 1,240 fathoms, 2 specimens. Station 78, 1,000 fathoms, 1 specimen. Station 50, 1,250 fathoms, 1 specimen.

I have not much question that this is Ljungman's *A. Otteri* which has some variety as to size and curve of spines. The unique originals of this and many other species were, with great kindness, lent me by Prof. Lovén; and Dr. G. O. Sars showed a similar generosity.

Amphiura depressa?

Amphipholis depressa Ljn. Öf. Kong. Akad. Oph. Viv., 1866, p. 312.

Station 233, 15 fathoms, 1 specimen.

Amphiura Studeri.

Amphiura antarctica Studer. Monatsb. Kön. Akad. Wissen., Berlin, 1876, p. 461.

Station 151, off Herd Isl., 75 fathoms, 1 specimen; var. Off Marion Isl., 50 – 75 fathoms, 10 specimens. Station 145, off Prince Edward's Isl., 310 fathoms, 1 specimen (young). Off Prince Edward's Isl., 85 – 150 fathoms, 1 specimen. Royal Sound, Kerguelen Isl., 28 fathoms, 12+ specimens. Balfour Bay, Kerguelen Isl., 20 – 60 fathoms, 8 specimens.

As I have combined *Amphipholis* with *Amphiura*, Prof. Studer's name has become a duplicate to (*Amphipholis*) *antarctica* Ljn. I take, therefore, the liberty of giving it the name of its discoverer, who kindly identified these specimens by his own.

Ophiocnida pilosa sp. nov.

Plate XII. Figs. 341 - 343.

Special Marks. — Disk scaling hidden. Disk set with stout simple spines. Five tapering arm-spines, the lowest one longest. A slender mouth-papilla on each side, and a pair of thick ones at apex of mouth-angle.

Description of an Individual (Station 162). — Diameter of disk 5.2 mm. Arm broken, but apparently eight or ten times the diameter of disk. Width of arm near disk 1.2 mm. The short narrow mouth-angle has at its base on either side a spiniform papilla, and at its apex a pair, stouter and more angular. Mouth-shields longer than broad, nearly oval. Side mouth-shields triangular, somewhat curved round the mouth-shield, not meeting within. Under arm-plates narrow, longer than broad, with eight sides, but having the angles rounded and nearly obliterated ; lateral sides re-enteringly curved. Side arm-plates feeble, nearly or quite meeting above, but not below. Upper arm-plates nearly twice as wide as long, of a transverse oval shape, with inner curve deeper than outer. Disk delicate but rather thick, sparsely set above and below with small spines ; in the centre may be seen some round, very thin, primary plates ; the rest seems naked, but on drying a very fine, delicate scaling appears. Radial shields much longer than broad, slightly curved, meeting without, widely separated within ; length to breadth 1 : .5. Five cylindrical, tapering, blunt arm-spines, the lowest somewhat the longest ; lengths to that of an under arm-plate, .5, .5, .5, .5, .7 : .5. No tentacle-scales. Color in alcohol, pale gray.

Station 162, 38 fathoms, 2 specimens. Station 212, 10 – 20 fathoms, 1 specimen.

Ophiocnida scabra sp. nov.

Plate XII. Figs. 344 - 346.

Special Marks. — Disk much puffed. Radial shields long and narrow. Five or six short stout arm-spines, the second longest. Two minute mouth-papillæ on either side, and a pair of larger ones at apex of mouth-angle.

Description of an Individual (Station 128). — Diameter of disk 6 mm. Length of arm about 40 mm. Width of arm near disk 1.3 mm. Two minute, bead-like papillæ on each side of base of small mouth-angle, and a pair, much larger, at its apex. Mouth-shields small, rounded, about as broad as long. Side mouth-shields small, bent, wider without than within, where they do not meet. Under arm-plates as broad as long, bounded by a curve without, and within by three sides of an octahedron. Side arm-plates narrow, widely separated above and below, and having a feeble spine-ridge. Upper arm-plates two and a half times as broad as long, of a clean, transverse oval shape. Disk extremely puffed in the interbrachial spaces by the swollen ovaries. This swollen portion, both above and below, is naked, and sparsely set with minute, peg-like spines ; but above the surface is finely and pretty uniformly scaled, with about 6 scales in the length of 1 mm. Radial shields long and very narrow, slightly bent towards each other, nearly or quite separated their whole length by a narrow strip of two scales ; length to breadth 1.5 : .3. Six short, thick, microscopically thorny arm-spines, whereof the two uppermost are longest, somewhat flattened, pointed, and have a minute beak ; those below diminish constantly in length, and are almost club-shaped ; lengths to that of a lower arm-plate, .5, .7, .4, .3, .3, .2 : .3. One round tentacle-scale. Tentacles papillose, as in *Ophiothrix.* Color in alcohol, pale yellowish-brown, mottled and speckled with darker.

Station 128, off Bahia, Brazil, 1,275 fathoms, 1 specimen.

This eccentric species might almost as well go with *Ophiactis*.

AMPHILEPIS Ljn.

Amphilepis patens sp. nov.

Plate XII. Figs. 338-340.

Special Marks. — Disk flat, round and smooth. Mouth-angle large with three wide mouth-papillæ on each side. Second pair of mouth-tentacles encircled by hard parts of the mouth.

Description of an Individual. — Diameter of disk, 11 mm. Width of arm near disk, 2 mm. Mouth-papillæ broad and irregular; on either side of the large prominent mouth-angle, at the outer corner, are two more or less closely joined; and, at the apex, a larger pair which, through the gap between them, show the small lowest tooth. Mouth-shields rather small, rounded, broader than long, often with a little peak inward; length to breadth, 1 : 1.2. Side mouth-shields short and wide; narrower within, where they barely meet. Under arm-plates, rather small, as broad as long, shield-shaped, with a gently curved outer side, lateral sides a little re-enteringly curved, and an obtuse angle within. Side arm-plates wide, with a knob-like spine-crest, meeting fully above and nearly or quite below. Upper arm-plates transverse oval, twice as wide as long, separated by the side arm-plates. Disk round and flat, but not thin; covered above and below with rounded, overlapping, flat, rather large, very thin, translucent scales, with indistinct outlines; above they are of pretty even size, except a marginal row of larger, each of which is .7 mm. long; below they are much finer; about 3 in the length of 1 mm. Radial shields large, of a rhomboidal form, except that the outer side is rectangular, much longer than wide, strongly diverging, with the outer ends nearly touching, but separated within by a broad wedge of numerous scales; length to breadth, 3 : 1.2. Three stout, short, cylindrical, tapering, blunt arm-spines; lengths to that of an under arm-plate, 1.1, 1.2, 1.3 : .8. Tentacle-pores large, with one minute scale on lateral side of under arm-plate. The roots of the second pair of mouth-tentacles come *low down*, and thus seem framed by the surrounding hard parts. Color in alcohol, pale gray.

Station 299, 2,160 fathoms, 1 specimen.

Amphilepis papyracea sp. nov.

Plate XVI. Figs. 429-431.

Special Marks. — Disk thin and flat, with thin, fine scales. Three tapering, rather slender arm-spines, a little longer than an arm-joint. No tentacle-scale. Radial shields nearly or quite separated their entire length.

Description of an Individual (Station 198). — Diameter of disk 9 mm.

Width of arm close to disk, without spines, 1.5 mm. Two wide, slender pointed mouth-papillæ on each side, standing high up on the jaws. Four teeth, the three upper ones flat and wide, with a curved cutting edge; the lowest thicker and more conical. Mouth-shields flat and small, of a wide heart-shape with a rounded angle inward and outer edge rounded; length to breadth .7 : 1. Side mouth-shields wide without, where they enclose the corner of the mouth-shield, narrow and just meeting within. Under arm-plates pentagonal with inner angle slightly truncated, lateral sides re-enteringly curved, and outer edge straight. Side arm-plates with outer edge swollen; meeting above, and nearly so below. Upper arm-plates thin and translucent, of a transverse oval shape, about twice as wide as long. Disk smooth, flat, angular and very thin, covered with small, thin, rounded, ill-defined scales. Radial shields with a vague outline, of a bent pear-seed shape, nearly touching without, separated within by an oval of five scales; length to breadth 2.5 : 1. Scaling on lower interbrachial space finer than that above. Three rather slender, bluntly pointed, tapering, cylindrical arm-spines, a little longer than an arm-joint, well up on the outer edge of side arm-plates. Tentacle-pores large, but without a scale. Color in alcohol, pale gray.

Station 198, 2,150 fathoms, 1 specimen.

Amphilepis tenuis sp. nov.

Plate XVI. Figs. 432–434.

Special Marks. — One minute tentacle-scale. One mouth-papilla on each side.* Radial shields short and wide, and joined for half their length.

Description of an Individual (Station 237). — Diameter of disk 4 mm. Width of arm close to disk, without spines, .7 mm. One wide, pointed, somewhat bent mouth-papilla high up on each side the mouth-angle, and a pair, short, thick, and rounded, at the apex. Mouth-shields small, twice as broad as long, of a transverse diamond-shape, with rounded angles. Side mouth-shields three-sided, short and swollen, wider without, tapering rapidly within, where they scarcely meet. Under arm-plates broad pentagonal, with a short angle within, outer side nearly straight, and laterals slightly curved. The first plate is large and of a truncated wedge-form. Side arm-plates meeting broadly above and nearly touching below. Upper arm-plates twice as broad as long, of a nearly semicircular outline, with the curve inward. Disk flat and angular, covered with very thin scales; in centre of the disk is a rosette of six large ill-defined primary plates, each nearly surrounded by minute scales. Radial shields short, wide pear-seed shaped, joined for the outer half of their length, narrowly separated within by a wedge of small scales. Scaling on interbrachial space below, much finer than that above. Three short, cylindrical, bluntly pointed arm-spines. One minute, rounded tentacle-scale, which easily falls off. Color in alcohol, faint greenish-gray.

Station 237, 1,875 fathoms, 1 specimen.

* Sometimes broken in two, as in the figure.

Amphilepis norvegica? Ljn.

Amphilepis norvegica. Öf. Kong. Akad. Oph. Viv., 1866, p. 322.

Station 45, 1240 fathoms, 1 specimen. Station 46, 1350 fathoms, 3 specimens. So far as one may judge, without having a proper series, these are the adult of Ljungman's original. They have the disk as large as 9 mm. The radial shields are pretty large and separated, and there is no tentacle-scale.

OPHIACTIS.

TABLE OF SPECIES HEREIN DESCRIBED.

Note. — Following these descriptions will be found the species previously known and brought back by the "Challenger," namely, *O. asperula*, *O carnea*, *O. Savignyi*, and *O Mülleri*.

Skin thick and much obscuring the scaling and mouth-shields. Radial shields narrow and small. Five short, thick, blunt, flattened arm-spines.	*O resiliens.*
Disk-scales distinct and naked, without spines. Three stout, blunt, tapering, cylindrical arm-spines. One large, flat mouth-papilla on each side. Teeth lobed. Five arms.	*O flexuosa.*
Disk-scaling coarse, and with few or no spines. Three or four stout, blunt, tapering arm-spines. Two or three mouth-papillæ on each side. Teeth lobed.	*O. cuspidata.*
Disk-scales coarse, and set with numerous short spines. Radial shields short and triangular. Four stout, cylindrical, tapering arm-spines. One mouth-papilla on each side. Five arms.	*O. nama.*
Disk finely scaled, and set with short, minute spines. Radial shields small and pear-seed shaped. Four moderately stout, tapering arm-spines, the uppermost longest. Two or three minute mouth-papillæ on each side. Seven arms.	*O. hirta.*
Disk-scales coarse and thick, with large radial shields. No spines, except a few near the margin. Four rather long and slender arm-spines, the upper one longest Two mouth-papillæ on each side.	*O. poa.*
Disk-scales larger in centre, where primary plates may be distinguished in a rosette. No spines, or only an occasional minute one on the margin. Three or four rather long and tapering arm-spines. Two mouth-papillæ on each side.	*O canotia.*

Of the above seven species, the first belongs with the shallow-water type of *O. Savignyi;* the rest come under the type of *O. Balli*, whose species often inhabit the deep sea.

Ophiactis resiliens sp. nov.

Plate XIII. Figs. 362 – 364.

Special Marks. — Skin thick and much obscuring the scaling and mouth-shields. Radial shields narrow and small. Five short, thick, blunt, flattened arm-spines.

Description of an Individual (Port Jackson). — Diameter of disk 6.5 mm. Length of arm 38 mm. Width of arm near disk 1.5 mm. Mouth-angle very small and short, carrying on either side two small, flat, squarish papillæ, and, at its apex, a third, rounded, with a minute point like the teeth. Mouth-shields small, of a transverse oval shape ; length to breadth .7 : .5. Side mouth-shields rather small and curved, broader without than within, where they meet. Under arm-plates small and rounded, about as long as broad, having outer side curved and inner side with ill-marked angles. Side arm-plates projecting in a strong spine-ridge. Upper arm-plates flat, transverse oval in form, about twice as broad as long. Disk covered below by a thick, naked skin, and above by

fine, crowded, irregular, thin scales, of the smallest of which there are about 5 in the length of 1 mm. Those near the radial shields are much larger; and there may be also obscurely distinguished six round primary plates, widely separated by the fine scaling. The disk margin is beset with minute, sharp, peg-like spines. Radial shields long and narrow, touching without, separated within by a narrow wedge of about three scales; length to breadth 1.3 : .4. Five short, thick, blunt, flattened arm-spines, of which the uppermost is the stoutest, but not longer than the rest. One oval tentacle-scale. Color in alcohol, above, olive green, mottled and banded with lighter; below, yellowish brown, with under arm-plates and arm-spines marked with orange.

Port Jackson, Australia, 30–35 fathoms, 1 specimen.

Ophiactis flexuosa sp. nov.

Plate XIII. Figs. 347–349.

Special Marks. — Disk-scales distinct and naked, without spines. Three stout, blunt, tapering, cylindrical arm-spines. One large flat mouth-papilla on each side. Teeth lobed. Five arms.

Description of an Individual (Station 171). — Diameter of disk 7 mm. Length of arm about 35 mm. Width of arm near disk 2.3 mm. Each side of the short, narrow mouth-angle is occupied by a single very large, wide, flat papilla, while a third, standing under and resembling the teeth, is at the apex, and has a rounded figure, with a decided peak or little lobe within. Mouth-shield somewhat broader than long, of a rounded diamond-shape. Side mouth-shields rather broad, wider without than within where they meet. First under arm-plate small, and wider within than without; those beyond are narrow compared with the width of the arm, much rounded, of a short transverse oval shape, with the inner side somewhat angular. Side arm-plates very wide, meeting neither above nor below, and having but a feeble lateral projection. Upper arm-plates broad and short, two and a half times as wide as long, of an elongated transverse diamond-form, sometimes with outer side so straight as nearly to be triangular. Disk without spines, and covered above with coarse, rounded, thick, overlapping scales, of which there are four or five radiating rows in the narrowest part of each interbrachial space. Below, the scales of the interbrachial space are much finer (4 or 5 in the length of 1 mm.), and regularly imbricated. Three short, stout, cylindrical, scarcely tapering arm-spines of nearly equal length, and about as long as one and a half joints; the upper spine stoutest. One large oval tentacle-scale. Color in alcohol, pale brown.

Station 171, 600 fathoms, 2 specimens. Station 142, 150 fathoms, 10 specimens, *young?*

The ten specimens from Station 142, 150 fathoms, may be the young of this species. They have six arms, while *O. flexuosa* has but five, and are scarcely to be distinguished from *O. plana;* and the question arises whether *O. plana* be not a young animal. The so-called adult of *O. Mülleri* has five arms, and the young six.

Ophiactis cuspidata sp. nov.

Plate XIII. Figs. 359-361.

Special Marks. — Disk-scaling coarse, and with few or no spines. Three or four stout, blunt, tapering arm-spines. Two or three mouth-papillæ on each side. Teeth lobed.

Description of an Individual (Station 170). — Diameter of disk 5 mm. Length of arm 25 mm. Width of arm close to disk 1.3 mm. Two large, broad, flat mouth-papillæ on each side, whereof the outer one is larger. Seven or eight large flat teeth, of a very wide heart-shape, and having a little lobe, or peak, within. Mouth-shields broader than long, wide heart-shape, or transverse diamond-shape, with rounded angles ; length to breadth .6 : .8. Side mouth-shields stout, slightly curved, rather broad, meeting within, where they have a rounded end. First under arm-plate stout and rather large, wider within than without, and having re-enteringly curved lateral sides. The plates beyond are shield-shaped, widest without, and having a somewhat obtuse angle within. Outer side curved, lateral sides re-enteringly curved. Side arm-plates stout, nearly meeting above and below, and having a well-marked spine-crest. Upper arm-plates broader than long, of a wide, transverse diamond-shape, with the outer angle much rounded. Disk thick and covered above with large, rather swollen scales, whereof there are three lines in each interbrachial space ; in the centre are six large, somewhat angular, primary plates, separated by single lines of much smaller angular scales ; the lower interbrachial space is covered with fine, thickened scales, from 5 to 8 in the length of 1 mm. Radial shields blunt pear-seed shape, swollen ; nearly or quite separated by a wide wedge of two or three scales. Along margin of disk are a few small, peg-like spines. Four stout, smooth, tapering, regular arm-spines, the upper one longest ; lengths to that of a lower arm-plate 1.7, 1.1, 1, .7 : .6. One stout, nearly oval tentacle-scale. Color in alcohol, pale gray.

Station 170, 520 fathoms, 5 specimens. Station 171, 600 fathoms, 1 specimen.

Ophiactis nama sp. nov.

Plate XIII. Figs. 350-352.

Special Marks. — Disk-scales coarse, and set with numerous short spines. Radial shields short and triangular. Four stout, cylindrical, tapering arm-spines. One mouth-papilla on each side. Five arms.

Description of an Individual (Station 174). — Diameter of disk 6 mm. Length of arm about 45 mm. Width of arm near disk 2.2 mm. One large, wide, flat mouth-papilla at base of mouth-angle on each side, and one (which may be called the lowest tooth) at the apex ; this last is broad and rounded, with a minute peak within. Mouth-shields of a much rounded, transverse diamond-shape ; length to breadth 8 : 1.1. Side mouth-shields stout, nearly meeting

without, broader without than within, where they touch. First under arm-plate small and three-sided, wider within than without: those beyond are one half broader than long, with a curved outer side, and an irregular, more or less truncated angle within. Side arm-plates unusually wide, but not much projecting, nearly meeting above and below. Upper arm-plates much wider than long, three-sided, with a faintly curved outer side, and an angle, sometimes truncated, within. Disk plentifully set with short, slender, cylindrical spines, and covered with well-rounded overlapping scales, which are large above (2 or 3 in the length of 1 mm.), and more regular and much smaller below (4 or 5 in 1 mm.). Radial shields sunken, rudely triangular, short and wide, separated by a broad wedge of three or four large scales; length to breadth 1.2 : 1. Four cylindrical, tapering, blunt, rather stout arm-spines, the two upper ones largest and somewhat longer than an arm-joint. One large, oval tentacle-scale. Color in alcohol, pale straw.

Station 174, 210–610 fathoms, 1 specimen. Station 171, 600 fathoms, 1 specimen.

Ophiactis hirta sp. nov.

Plate XIII. Figs. 365–367.

Special Marks. — Disk finely scaled, and set with short, minute spines. Radial shields small and pear-seed shaped. Four moderately stout tapering arm-spines, the uppermost longest. Two or three minute mouth-papillæ on each side. Seven arms.

Description of an Individual (Station 164^a). — Diameter of disk 4.3 mm. Length of arm about 14 mm. Width of arm near disk 1.2 mm. Two or three small, narrow, scale-like mouth-papillæ on either side of the very narrow mouth-angle; and one, wide, flat, and pointed, at the apex; this last may, as in all similar cases, be considered the lowest tooth. Mouth-shields small, of a much rounded diamond-shape; sometimes nearly circular. Side mouth-shields narrow, of nearly equal width, meeting within. Under arm-plates rather small, as broad as long, bounded without by a strong curve, and within by three sides of an octagon. Side arm-plates stout, projecting laterally in a well marked spine-ridge, meeting neither above nor below. Upper arm-plates a little broader than long, transverse oval, with the inner sides more or less angular. Disk covered with coarse, thickened, irregular scales, those of the under surface being sometimes wholly obscured by a thick skin; those in the centre are largest, but the primary plates are not readily distinguishable; there are small, peg-like spines scattered over the entire surface. There are seven pairs of radial shields, which are small, sunken below the disk-surface, of a blunt pear-seed shape, and separated by a rather wide wedge of three scales. Four smooth, rounded, tapering, moderately stout arm-spines; the upper one longest; lengths to that of an under arm-plate, 1, .8, .7, .7 : .5. One stout, oval tentacle-scale. Color in alcohol, gray mottled with pale brown.

Station 164^a, 400 fathoms, 1 specimen.

Ophiactis poa sp. nov.

Plate XIII. Figs. 356-358.

Special Marks. — Disk-scales coarse and thick, with large radial shields; no spines except a few near the margin. Four rather long and slender arm-spines, the upper one longest. Two mouth-papillæ on each side.

Description of an Individual (off Tristan d'Acunha). — Diameter of disk 5 mm. Length of arm about 30 mm. Width of arm near disk 1 mm. On each side of the short narrow mouth-angle are two rather large, squarish, flat papillæ, of which the outer one is broader; at the apex is usually a very small heart-shaped papilla, similar in shape to the larger teeth above it. Mouth-shields much wider than long, of a rounded transverse heart-shape; the inner sides a little re-enteringly curved. Side mouth-shields of nearly equal width, meeting broadly within. Under arm-plates wide shield-shaped, bounded without by a broad curve, within by an obtuse or truncated angle, and on the lateral sides by re-entering curves. Side arm-plates nearly meeting above and below, not very wide, but projecting in a well-marked spine-crest. Upper arm-plates broader than long, fan-shaped with an obtuse angle inward. Disk covered with coarse, overlapping scales; those below regular and smaller, about 4 in the length of 1 mm.; those above much larger and more irregular; in the centre an irregular rosette of large, rounded plates, and in each interbrachial space about three radiating rows of elongated scales. The disk margin is sparsely set with small peg-like spines. Radial shields large, of an angular pear-seed shape, separated wholly by a narrow wedge of two or three scales; length to breadth 1.5 : 1. Four slender, cylindrical tapering arm-spines, the uppermost longest; lengths to that of an under arm-plate 1.2, .8, .8, .8 : .5. One large, oval tentacle-scale. Color in alcohol, pale gray.

Off Tristan d'Acunha, 1,000 fathoms, 2 specimens. Off Tristan d'Acunha, 500 fathoms, 10 specimens. Both Station 135.

Ophiactis canotia sp. nov.

Plate XIII. Figs. 353-355.

Special Marks. — Disk-scales larger in centre, where primary plates may be distinguished in a rosette; no spines, or only an occasional minute one on the margin. Three or four rather long and tapering arm-spines. Two mouth-papillæ on each side.

Description of an Individual (Station 73). — Diameter of disk 5.5 mm. Length of arm about 17 mm. Width of arm near disk 1.8 mm. Two flat, rather large, squarish mouth-papillæ on each side of the narrow mouth-angle, and one at the apex, similar in form to the teeth, which are broad heart-shape with a peak within. Mouth-shields wider than long, broad heart-shaped with a rounded angle within, or wide transverse, rounded diamond-shaped. Side mouth-shields rather narrow, of about equal width, meeting fully within. First under arm-plate small and wider within than without; those beyond are wide shield-shaped, bounded without by a curve, on the lateral sides by re-entering

curves, and within by an obtuse or truncated angle. Side mouth-shields of moderate width, nearly meeting above and below, and having a well-marked spine-crest. Upper arm-plates broad, transverse diamond-shaped, with outer and inner angles rounded. Disk covered with rather thick overlapping scales, which are finest below, near the mouth-shields, where there are about 7 in the length of 1 mm. Above, the centre is occupied by a rosette of two circles of large rounded plates partially separated by a few small scales. Radial shields short, wide pear-seed shaped, separated their entire length by a narrow wedge of three scales. On interbrachial spaces below, a few minute, peg-like spines. Four short, cylindrical, tapering, blunt arm-spines, all stout, especially the lower ones; upper spine longest, and about as long as one and a half joints. One large oval tentacle-scale. Color in alcohol, pale straw.

Station 73, 1,000 fathoms, 2 specimens.

Ophiactis asperula Ltk.

Ophiactis asperula. Addit. ad Hist. Oph., Pt. II., 1859, p. 130.

Ophiactis magellanica Ljn. Öf. Kong. Akad. Oph. Viv., 1866, p. 325.

Station 308, 175 fathoms, 1 specimen. Station 311, 245 fathoms, 1 specimen. Station 312, 10–15 fathoms, 12+ specimens. Station 315, 5–12 fathoms, 7 specimens.

Ophiactis carnea Ljn.

Ophiactis carnea. Öf. Kong. Akad. Oph. Viv., 1866, p. 324.

Station, Simon's Bay, Cape of Good Hope, 10–20 fathoms, 4 specimens.

Ophiactis Savignyi Ljn.

Ophiactis Savignyi. Öf. Kong. Akad. Oph. Viv., 1866, p. 323.

Station 208, 18 fathoms, 1 specimen. Zanzibar, 10 fathoms, 2 specimens.

Ophiactis Mülleri Ltk.

Ophiactis Mülleri. Vid. Meddel., Jan. 1856, p. 12.

Off Bahia, Brazil, 7–20 fathoms, 2 specimens, var. *quinqueradia.* Station 122, 350 fathoms, 2 specimens.

OPHIOSTIGMA Ltk.

Ophiostigma africanum.

Plate XIII. Figs. 368–370.

Special Marks. — Arms more than eight times the diameter of disk. Outer mouth-papillæ very wide. Radial shields long, narrow, and joined.

Description of an Individual (Cape de Verde Isl.). — Diameter of disk 2.2 mm.

Length of arm 18 mm. Width of arm near disk .6 mm. Three mouth-papillæ on each side of a mouth-angle, whereof the two inner ones are small, short, and almost conical, while the outer is straight and very wide, extending from the first under arm-plate about two thirds the length of an angle. Mouth-shields three-sided, with rounded angles, bounded without by a curve, and within by a rounded angle; length to breadth, .2 : .3. Side mouth-shields wide, a little broader without than within, where they fully meet. Under arm-plates small, pentagonal, with outer side nearly straight, lateral sides a little re-enteringly curved, and an angle within. Side arm-plates nearly meeting above and below, and having a thick, low, spine-crest. Upper arm-plates small, irregular transverse oval, with the inner curve deeper than the outer. Disk rather thick, standing nearly clear of the arms, as is usual in the genus: covered with fine, thin, nearly equal, indistinct scales, whereof most are rounded, but some, near the centre, are angular: there are about 12 in the length of 1 mm. where they are finest. Along margin of disk are minute, peg-like, scattered spines, which are not jointed at the base. Radial shields long, narrow, and closely joined; length to breadth, .6 : .2. At their outer ends are visible the points of the genital plates, in two little lobes. Three stout, equal, peg-like, very short arm-spines, standing nearly at right angles with the arm. Two minute, longer than broad tentacle-scales standing diagonally with the arm-plate. Color in alcohol, nearly white.

St. Vincent, Cape de Verde Islands, 7 specimens.

O. africanum differs from *O. isacanthum* in having longer arms, and longer, narrower radial shields; and from *O. formosa* by its wide outer mouth-papilla and longer arms.

OPHIOPHOLIS Müll & Trosch.

Ophiopholis japonica sp. nov.

Plate XIII. Figs. 374-376.

Special Marks. — Upper disk covered with thin scales and large radial shields, neither of which have grains or spines, except the marginal scales. Five stout, cylindrical, tapering arm-spines.

Description of an Individual (Station 236). — Diameter of disk 10 mm. Length of arm about 40 mm. Width of arm without spines near disk 2.7 mm. Three or four small, irregular, flat, scale-like mouth-papillæ on each side, and a flat clump of short, bead-like tooth-papillæ at apex of mouth-angle. Mouth-shields and side mouth-shields somewhat obscured by thick skin. The former are transverse oval, much wider than long; length to breadth .8 : 1.3. Side mouth-shields small and short, with rounded ends, rather wider within than without, and somewhat bent. Under arm-plates a little wider than long, slightly separated, and with rounded corners. Side arm-plates closely soldered with their neighbors, meeting neither above nor below, rising laterally

in a strong spine-ridge. Upper arm-plates transverse oval, twice as broad as long, slightly swollen, each surrounded by a single line of rounded granules, which are broader than long. Disk round and thick, with a flat top, covered with thin, variously shaped scales, which, near the margin, are obscured by thick skin; those of the centre small, round, and arranged in a rosette; those farther out, larger and elongated, arranged in three or four rows between the radial shields in the interbrachial spaces, where they are beset with a few scattered grains, which at the margin become much more numerous and larger, and appear as very short spines. Interbrachial spaces below covered with a few grain-like spines. Radial shields large, pear-seed shaped, much longer than wide, separated usually by a line of two large and two small scales. Genital openings large and extending about two thirds the distance to the margin. Five, rarely six, stout cylindrical, blunt, tapering arm-spines, whereof the second and third are stoutest, and as long as one and a half arm-joints. One, and on the first two joints sometimes two, small, rounded tentacle-scales. At tip of arm are four slender spines, of which the lowest takes the form of a flat, long, three-toothed hook, as elsewhere in this genus. Color in alcohol, above, light pink; below, pale straw.

Prof. P. Martin Duncan has recently published (Linnean Soc. Journ. Zoöl., XIV. 460, 479) an Ophiuran, *Ophiolepis mirabilis*, of which he remarks: "This common species has the disk of *Ophiolepis* as diagnosed by Müller and Troschel, that is to say, the scales, which are of good size, and the large radial shields, are environed by rows of small scales as by belts. But the upper arm-plates have also the supplementary rows of small scales around them, and there are also large accessory side pieces. Moreover, there are hooks on the side arm-plates. This mixture of Ophiolepian and Ophiopholian characters is very interesting; and this species, I consider, renders the abolition of *Ophiopholis* as a genus inevitable."

The meaning of this passage is not quite clear, because Müller and Troschel (Syst. d. Asterid., p. 89) diagnosed, not the whole genus, but only the first section of it, as having belts of scales round the disk plates (e. g. *O. cincta*). To this section *Ophiolepis* has been restricted. The third section they described as having spines on the scales. This last is *Ophiopholis*, a genus now recognized as quite remote from the true *Ophiolepis*, which stands nearer *Ophioglypha*, *Pectinura*, &c., while *Ophiopholis* approaches the *Amphiuræ* through *Ophiactis asperula*. It is evident that *Ophiolepis mirabilis* is a true *Ophiopholis*, lacking none of its characters, and standing quite near the typical *O. aculeata*. The fact that certain small scales surround larger ones is not here of generic importance, and probably results from the young stage of the specimen, which, to judge from the figures, had a disk not exceeding 4 mm. in diameter. *Ophiopholis japonica* differs from the old species as well as from *O. mirabilis* in its more slender arm-spines, and in having the radial shields and much of the upper disk free of grains or spines.

Station 235, 565 fathoms, 1 specimen. Station 236, 420–775 fathoms, 3 specimens.

OPHIOCHONDRUS Lym.

Ophiochondrus stelliger.

Plate XIII. Figs. 371-373.

Special Marks. — Disk finely and evenly granulated on both sides. Four slender arm-spines, whereof the uppermost is much the longest.

Description of an Individual (Station 320). — Diameter of disk 5 mm. Length of arm 16 mm. Width of arm near disk 1.3 mm. Three mouth-papillæ on each side, whereof the two outer are flattened and squarish, while the innermost is stout, rounded, tapering, and peg-like. Apex of mouth-angle occupied by the lowest tooth, which sometimes is represented by two blunt, spiniform papillæ similar to their next neighbor. Four rather narrow teeth, which sometimes are almost spiniform, but usually are flattened. Mouth-shields much wider than long, with a well-marked obtuse angle inward and the outer side gently curved ; length to breadth, .7 : 1.1. Side mouth-shields long, rather narrow, of nearly equal width, slightly curved, and fully meeting within. First under arm-plate small, longer than broad, hexagonal, with rounded corners ; the plates beyond are rather small, wider than long, bounded without by a broad curve, and within by an obtuse angle ; the lateral sides are very short, or are confounded in the outer curve. Side arm-plates small, somewhat wider than long, fan-shaped, with inner angle rounded. Disk rather thick, finely and uniformly granulated above and below, about 17 grains in the length of 1 mm. Four cylindrical, tapering, rather slender arm-spines, whereof the uppermost is longest : lengths to that of an arm-joint, 1.1, .6, .5, .4 : .6. One small, narrow tentacle-scale. Color in alcohol, straw.

Station 320, 600 fathoms, 7 specimens.

OPHIOCONIS Ltk.

Ophioconis antarctica sp. nov.

Plate XIV. Figs. 380-382.

Special Marks. — Seven slender, cylindrical, tapering arm-spines, the two upper ones longest. One large tentacle-scale. Disk closely granulated, except mouth-shield ; 5 or 6 grains in the length of 1 mm.

Description of an Individual (Station 150). — Diameter of disk 13 mm. Length of arm about 60 mm. Width of arm at base, without spines, 2 mm. There are to each angle of the mouth twelve or fourteen papillæ, of which the innermost are slender and pointed, while the outer one on either side is broad and squarish ; at the apex there is a cluster of four or five, which properly might be called tooth-papillæ. Five or six rather narrow, flat, blunt teeth, whereof the lowest is often split in two. Mouth-shields broad triangular, with a blunt angle inward and outer edge nearly straight ; they are more or less obscured by granules, which completely hide the side mouth-shields. These

are small, longer than wide, and broader without than within, where they nearly or quite meet. Under arm-plates much broader than long, pentagonal, with a blunt inner angle, outer edge slightly curved, and laterals re-enteringly curved. Side arm-plates somewhat projecting, nearly meeting below, but well separated above by the thick, broad, somewhat arched upper arm-plates, which are wide fan-shaped, with a blunt angle inward. Under the microscope they appear minutely tuberculous, while the lower plates are ornamented with wavy lines. Disk thick and nearly round, completely covered with coarse, rounded granules, 5 or 6 in the length of 1 mm. on the upper surface, and more scattered below. The underlying scales are extremely thin and smooth. Genital openings long, extending from outer corners of mouth-shield nearly or quite to the margin of disk. Seven long, smooth, cylindrical, tapering arm-spines, the two upper ones as long as three or four arm-joints ; the others somewhat shorter. One long, wide tentacle-scale, with a rounded point occupying the lateral side of the under arm-plate. Color in alcohol, nearly white.

Station 150, 150 fathoms, 12+ specimens. Off Prince Edward Isl., 85–150 fathoms, 12+ specimens. Off Marion Isl., 50–75 fathoms, 12+ specimens.

Ophioconis pulverulenta sp. nov.

Plate XIV. Figs. 377–379.

Special Marks. — Disk finely, closely, and evenly granulated, with about 14 grains in the length of 1 mm. Eight or nine long, delicate, somewhat flattened arm-spines, the three uppermost longest, and nearly equal. Two tentacle-scales.

Description of an Individual (Station 172). — Diameter of disk 12 mm. Length of arm about 55 mm. Width of arm close to disk, without spines, 3.2 mm. Ten small, short, close-set, pointed mouth-papillæ on each side of the mouth-angle, and one somewhat stouter at the apex ; the two outermost are broadest and most rounded. Mouth-shields large, as broad as long, of a rounded heart-shape. Side mouth-shields stout and wide, broader without than within, where they do not meet. Both they and the mouth-shields are more or less covered by a granulation, which, as well as that of the disk, is liable to be rubbed off. Under arm-plates axe-shaped, much broader without, where the edge is curved, and with deep re-entering curves on the lateral sides. Side arm-plates thin and microscopically corrugated. Upper arm-plates thin, with a central ridge, about twice as broad as long, much wider without than within, with sharp outer lateral corners and straight sides. Disk round and quite thick closely and evenly covered with minute granules, 12 or 14 in the length of 1 mm. Underneath these granules there are fine uniform, overlapping scales, about 5 in the length of 1 mm., among which the radial shields cannot be distinguished. Eight or nine long, slender, tapering, flattened arm-spines, whereof the three uppermost are about 2.3 mm. long and nearly equal, and the other five or six from 2 mm. to 1.7 mm. long. Two long, thin, nearly oval tentacle-scales, which are two thirds as long as an under arm-plate. Color in alcohol, pale straw.

Station 172, 240 fathoms, 1 specimen.

This species stands very close to *O. miliaria* of the West Indies, and comes from a similar depth. It seems sufficiently distinguished by the arm-spines, which are more numerous by one or two, and more flattened, showing even a feeble tendency to become spatulate.

OPHIOMYCES Lym.

Ophiomyces grandis sp. nov.

Plate XIV. Figs. 383 - 385.

Special Marks. — Eleven sharp, flat arm-spines, set along the whole upper and side edge of the plate, and growing longer from above down to the ninth. Basal under arm-plates, large and squarish, and bearing three long spatula-like tentacle-scales.

Description of an Individual (off Tristan d'Acunha). — Diameter of disk 6.5 mm. Length of arm about 25 mm. Width of arm near disk 2.2 mm. Four or five broad, flat teeth, with a curved, cutting edge; the lowest one being much the narrowest. Below these, and still on the jaw-plate, are three spiniform tooth-papillæ. Then, from apex of mouth-angle, there radiate, on each side, two rows of long, flattened mouth-papillæ, which completely hide the underlying parts; each row has five or six papillæ, of which the innermost one is spiniform, resembling a tooth-papilla; those beyond, more or less spatula-shaped, grow progressively larger and wider, until the outermost has almost a fan-shape; all incline more or less downward and outward, so that they overlap, tile fashion. On cutting away the mouth-papillæ, a small mouth-shield, of an irregular, short diamond-shape, may be seen, together with small triangular side mouth-shields, which nearly meet within. Length of mouth-shield to breadth .7 : .7. The jaws are long, narrow, and slender, with very large sockets at their base for the second pair of mouth-tentacles. The first under arm-plate is minute, triangular, and difficult to distinguish; the second very narrow, closely soldered with surrounding parts, and with deep re-entering curves on the lateral sides; the fourth plate is four-sided, about as broad as long, much wider without than within, and with deep re-entering curves on the lateral sides; length to breadth .6 : .7. Side arm-plates separated below, meeting narrowly above, not swollen, but clean cut and flaring outward. Upper arm-plates twice and a half as broad as long, shaped like segments of a circle, with a clean curve outward; near tip of arm they are nearly as long as wide, and form a pointed curve, while the side arm-plates are but slightly flaring and meet above on a line as long as the upper plate. The disk was much torn (as usually is the case), but evidently was covered above and below with fine scales, about 4 in the length of 1 mm., whereof many bore minute, peg-like spines. Eleven arm-spines, increasing rapidly in length from the first to the ninth, then diminishing; the upper ones are slender, sharp, and little flattened; the lower ones are broad, flat, sharp, and shaped like a bronze sword; lengths to

that of an under arm-plate, .2, .3, .3, .3, .5, .7, .8, 1, 1.2, .7, .7 : .7. The basal under arm-plates, as far as the fifth or sixth, bear on each lateral side three long, flat, spatula-like tentacle-scales, which project over the pore; for some distance beyond there are but two such scales, while a third, trowel-shaped, stands on the edge of the side arm-plate. One third out on the arm there remains only the large trowel-shaped scale. Color in alcohol, pale gray.

Station, off Tristan d'Acunhâ, 1000 fathoms, 2 specimens.

The peculiar twisting upward of the arms and disk of *Ophiomyces* is explained by the *absence of radial shields*, a want not yet observed in any other genus. It seems, then, that one function of radial shields is to keep the disk in shape, somewhat like the action of the sticks of an umbrella.

Ophiomyces spathifer.

Plate XIV. Figs. 386-388[a].

Special Marks. — Outer mouth-papillæ large and paddle-shaped. One flat, rounded tentacle-scale. Ten flattened arm-spines of various shapes, whereof the two lowest are borne on the under arm-plate.

Description of an Individual (Station 235). — Diameter of disk 3.5 mm. Width of arm next disk 1.2 mm. Three short, narrow, slightly flattened, peglike teeth, carried on a thick, lumpy jaw-plate, which also bears two long, flat, narrow, spatula-like tooth-papillæ. On either side of the mouth-angle are two radiating rows, each of about six long, flattened papillæ, which are imbricated and point downward and outward, so that the entire mouth-angle is hidden by them; the inner ones are narrow and spatula-like, but outwards they grow rapidly larger, so that the outermost are wide paddle-shaped, or even fan-shaped, their length to extreme breadth being .7 : .5. Mouth-shields shaped like a long, sharp, narrow lance-head. Side mouth-shields three-sided, delicate, separated as by a wedge by the mouth-shield, which extends inward considerably beyond them. Within, and indistinctly separated from the side mouth-shields, project the long jaws. These parts are all hidden, and can be seen only by cutting away the mouth-papillæ. Under arm-plates small, with re-enteringly curved lateral sides, wider without, where they are a little swollen, than within, separated by the side arm-plates, which meet narrowly both above and below, and are highest and most flaring at their outer edge. Upper arm-plates minute (sometimes apparently wanting), twice as long as broad, and appearing like little swellings just outside the juncture of the side arm-plates. The larger part of upper surface of arm is thus left uncovered, so that the arm-bones and their muscular bundles may be seen. Disk (as usual in the genus) distorted and pushed upward, covered uniformly with minute, thin, translucent, flat scales, without spines; there are about 13 in the length of 1 mm. Ten arm-spines, of which the three highest are equal, slender, narrow and tapering, and as long as any; the next two are of about the same length, but broad and flat, with rounded ends; the next three similar, but shorter; the two lowest spatula-like, with ends cut square off, and carried, not on the side arm-plate, but widely spaced on the

outer part of the *under* arm-plate ; lengths to that of an arm-joint, .5, .5, .5, .5, .5, .4, .4, .3, .3, .3 : .5. One flat, short, wide tentacle-scale, broader without than within, and, like many of the arm-spines and mouth-papillæ, microscopically striated. Color in alcohol, disk, gray ; arms, straw.

Station 235, 565 fathoms, 3 specimens in bad condition.

PECTINURA Forbes.

Pectinura arenosa sp. nov.

Plate XIV. Figs. 392-394.

Special Marks. — Nine to eleven short arm-spines. Disk uniformly granulated, with about 8 grains in 1 mm. long. No water-pores between under arm-plates.

Description of an Individual (Station 162). — Diameter of disk 10 mm. Length of arm about 42 mm. Width of arm close to disk 2 mm. Fifteen short, stout, pointed, crowded mouth-papillæ, the three outermost being somewhat the widest. Mouth-shields rounded triangular, about as broad as long, with a blunt angle inward and outer side straight. Supplementary shield semicircular, and about two thirds as large as the true shield. Side mouth-shields very small, and short, occupying part of the outer angles of mouth-shield, and widely separated within. First under arm-plate wide and large, and nearly semicircular though the inner side is not quite straight ; those beyond are as broad as long. There are no water-pores between the plates. Side arm-plates flat and not swollen, separated above and below. Upper arm-plates short rounded oval ; somewhat broader than long. Disk somewhat angular and slightly swollen, closely covered above and below, except the mouth-shields and side mouth-shields, with a fine granulation, about 8 grains in the length of 1 mm. Genital openings extending from mouth-shield about two thirds the distance to the margin. Nine to eleven short, stout, somewhat flattened peg-like arm-spines, all about half as long as the side arm-plate, except the lowest, which equals it. Two small rounded tentacle-scales on the side arm-plate, whereof that on the interbrachial side overlaps the base of the lowest arm-spines. Color in alcohol, disk pale yellowish brown, above ; arms darker, with irregular belts of black and yellowish brown.

Station 162, 38 fathoms, 6 specimens. This species stands between *P. spinosa* and *P. infernalis.*

Pectinura heros sp. nov.

Plate XIV. Figs. 389-391.

Special Marks. — Three very short arm-spines, low down on the side arm-plate. No pores between lower arm-plates.

Description of an Individual (Station 191). — Diameter of disk 22 mm. Length of arm about 100 mm. Width of arm close to disk without spines

4 mm. Fifteen small, close-set mouth-papillæ to each angle, whereof the two or three outer ones on each side are flat, rounded, and larger than the rest, which are pointed ; there are two just under the teeth, and sometimes two supplementary below and outside these. Mouth-shields long heart-shaped, with a rounded angle within ; length to breadth 3 : 2.2. Sometimes a rudimentary supplementary piece may be seen, just outside. Side mouth-shields three-cornered and small, occupying only the outer corners of the mouth-shield. Under arm-plates about as wide as long, bounded without by a curve, within by a truncated angle, and laterally by re-entering curves. Side arm-plates short, with rounded edges, meeting neither above nor below. Upper arm-plates broad, highly arched, closely overlapping, with outer and inner edges nearly straight. Disk flat and angular, closely and evenly covered with very fine granules, 7 or 8 in the length of 1 mm., except the radial shields and one or more plates along the margin. Radial shields egg-shaped, longer than broad, with outer and inner ends much rounded ; length to breadth 3.7 : 2. Lower interbrachial space covered by same granulation as above, extending even to the mouth-angle, but not on mouth-shields. Genital opening long, extending from mouth-shield to margin of disk. Three short, small, blunt arm-spines standing low on the side arm-plate, and about half as long as a joint. One round tentacle-scale. Color in alcohol white.

Station 191, 800 fathoms, 1 specimen.

This species stands as near *P. stellata* as to any ; there are, however, no pores between the under arm-plates, and but three short arm-spines. The only occasional presence of rudimentary supplementary mouth-shields points once more to the very close connection between *Ophiopeza* and *Pectinura*.

Pectinura maculata Vll.

Pectinura maculata. Proc. Bost. Soc. N. H., XII., 1869, p. 388.

Queen Charlotte's Sound, New Zealand, 10 fathoms, 5 specimens.

Pectinura rigida Lym.

Pectinura rigida. Bull. Mus. Comp. Zoöl., III. 10, 1874, p. 224.

Fiji Islands, 2 specimens.

Pectinura stellata Ltk.

Pectinura stellata. Addit. ad Hist. Oph., Pt. III., 1869, p. 33.

Station 208, 18 fathoms, 1 specimen.

Pectinura gorgonia Ltk.

Pectinura gorgonia. Addit. ad Hist. Oph., Pt. III., 1869, p. 33.

Fiji Islands, 1 specimen.

OPHIOPEZA Peters.

Ophiopeza aster sp. nov.

Plate XIV. Figs 395-397.

Special Marks. — Disk densely and finely granulated above and below, including the mouth-angle.

Description of an Individual (Station 142). — Diameter of disk, 11 mm. Length of arm, 33 mm. Width of arm close to disk, 2 mm. Teeth narrow, sharp and lanceolate ; the two lowest usually split in two. The apex is occupied by a bunch of three or four short, crowded, spiniform tooth-papillæ ; and on each side of the mouth-angle is a close line of small mouth-papillæ, whereof the inner ones are bead-like, while the two outermost are wider and somewhat flattened. The small, rounded mouth-shields and the side mouth-shields are completely covered by a close granulation. First under arm-plate about half as large as those beyond, of a heart-shape, with the point inward ; the rest are rather small, somewhat broader than long, much wider without than within, having the outer side curved, lateral sides re-enteringly curved and a truncated angle within. Side arm-plates small, clinging close to arm, widely separated above, nearly meeting below. Upper arm-plates four-sided, twice as broad as long, much wider without than within, with outer side gently curved and laterals straight. Disk pentagonal, flat, densely and uniformly covered with an extremely fine granulation, 20 or 25 grains in the length of 1 mm. ; this granulation extends over the entire mouth angle quite to the bases of the mouth-papillæ. Six very short arm-spines, growing longer from above downward ; the upper ones are rounded and peg-like ; the lowest ones somewhat flattened, and scarcely more than half as long as a joint. One oval tentacle-scale. Color in alcohol, light greenish gray.

Station 142, 150 fathoms, 6 specimens.

OPHIOTHRIX Müll. & Trosch.

Ophiothrix aristulata sp. nov.

Plate XV. Figs. 421-424.

Special Marks. — Ten moderately stout, feebly thorny, scarcely tapering arm-spines. Disk, except the large radial shields, densely set with short, slightly rough spines.

Description of an Individual (Station 142). — Diameter of disk 14 mm. Width of arm near disk 3 mm. There are about thirty tooth-papillæ which are pointed, and are arranged, as usual, in a vertical oval, the exterior line on either side composed of ten or eleven longer ones, while a similar number of shorter ones, arranged in twos at the centre, and in a single line above and below, fill closely the middle space. Three short, thick, squarish teeth. Mouth-

shield well marked, of a transverse diamond-shape, with rounded corners. Side mouth-shields thick and slightly swollen, rather wide, nearly or quite meeting within, tapering gently inward. Under arm-plates somewhat wider than long, with a wide curve without, short re-enteringly curved laterals, and straight inner laterals sloping towards the median line. Side arm-plates presenting a moderately prominent spine-crest. Upper arm-plates wider than long, slightly overlapping, of a transverse diamond-shape, with corners rounded or truncated; each plate has a median ridge, which gives to the upper arm a carinate look. Disk thick and strongly lobed in the interbrachial spaces; its upper surface occupied chiefly by large radial shields, which are long triangular, with a length to breadth of 5 : 3; they unite without, where each has a lobe projecting over the arm, separated within by a narrow wedge of scales bearing one or two rows of short, slightly rough spines: similar but somewhat longer spines densely clothe the centre and interbrachial spaces, passing over the margin and investing the outer portion of the naked surface below; the longest spines are 1.7 mm. Ten moderately stout, scarcely sapering, somewhat flattened, translucent arm-spines, bearing feeble thorns on their edges; the uppermost and lowest are minute, the rest diminish in length from the third downward; lengths to that of an under arm-plate, .8, 3.6, 4.6, 3.6, 3, 3, 2.6, 2, 1, .8 : 1. The first tentacle-pore has no scales; those beyond have a minute lip-like one in the angle of the under and side arm-plates. Color in alcohol, above, pale purplish pink, the side arm-plates and outer edges of radial shields marked with darker; below, much paler.

Station 142, 150 fathoms, 12+ specimens. Station 161 (*var.* with coarser spines), 38 fathoms, 2 specimens. Station 163 (*var.*), 120 fathoms, 5 specimens.

The species is readily distinguished from *O. capensis* by lacking the black stripe on the arm, and by having arm-spines serrated their whole length.

Ophiothrix capillaris sp. nov.

Plate XIV. Figs. 401-404.

Special Marks. — Very large, with nine very delicate, translucent arm-spines, whereof the upper ones are extremely long. Disk set with minute stumps, which are few and scattered on the large radial shields.

Description of an Individual (Station 204). — Diameter of disk 22 mm. Width of arm near disk, 4.8 mm. The vertical oval has over fifty tooth-papillæ of various sizes, those in the lower half being minute, crowded, and grain-like, while those on the margin of the upper half are large and thick, and project beyond the median papillæ. Four flat teeth, with rounded cutting edge; the uppermost and lowest narrowest. Mouth-shields small, much broader than long, bounded by a gentle curve without and an obtuse angle within; length to breadth .8 : 1.8. Under arm-plates small, narrow, about as long as broad, eight-sided, with angles more or less rounded and lateral sides a little re-enteringly curved. Side arm-plates with a well-marked spine-ridge. Upper arm-

plates about as broad as long, of a short diamond-shape, with angles rounded, rising on the median line in a low ridge and microscopically tuberculous. Disk round and flat, scarcely lobed in interbrachial spaces, more or less closely beset above and below with minute stumps bearing an irregular crown of thorns; on the radial shields they are much more scattered, smaller, and less thorny, and next the genital openings there are none. The radial shields, whose outlines are distinguishable through their covering, are triangular and very large, with a small lobe where they unite over the arm ; inwardly they diverge, and sometimes again bend together so as nearly or quite to reunite; length to breadth 9 : 4.5. On joints next disk there are nine slender, glassy, translucent, slightly flattened feebly thorny spines, whereof the uppermost are extremely long and elegant; those below progressively shorter ; lengths to that of an under arm-plate, 15.5, 15, 13, 9, 7, 6, 5, 3, 1.7 : 1.7. One small, blade-like tentacle-scale in the angle of the under and side arm-plates. Color in alcohol, above, pale brownish pink ; below, very pale yellowish brown ; along upper side of arm is a wide, brown stripe, whose edges are darkest.

Station 204, 100 – 115 fathoms, 3 specimens. Cebu ; 100 fathoms.

O. capillaris belongs near *O. comata* and *O. Suensonii.* It has an arm-stripe like that of the former, but has little stumps on the disk instead of hair-like spines.

Ophiothrix berberis sp. nov.

Plate XV. Figs. 425 - 428.

Special Marks. — Seven short, blunt, much flattened, strongly toothed arm-spines. Radial shields and interbrachial spaces below nearly or quite naked. Rest of disk set with short stumps bearing a crown of thorns.

Description of an Individual (Station 192). — Diameter of disk 9 mm. Width of arm near disk 2.5 mm. Length of arm about 58 mm. The vertical oval has about seventeen stout, blunt, nearly equal tooth-papillæ, whereof the marginal ones are scarcely longer than those in the middle. Three squarish, rather thin teeth. Mouth-shields broader than long, with an obtuse angle inward and a gentle curve without ; length to breadth, 1 : 1.5. Side mouth-shields rather narrow, slightly swollen, wider without than within, where they scarcely meet. First under arm-plate unusually large, nearly equalling the second, squarish, with rounded corners and an obtuse angle within. The plates increase in size to the seventh, which is broader than long, bounded without by a wide curve, and within by a truncated angle ; length to breadth .7 : 1.1. Side arm-plates furnished with a low thick spine ridge. Upper arm-plates transverse diamond-shaped, overlapping, having outer angle rounded and inner one truncated ; length to breadth .7 : 1.4. Disk rather flat, lobed in the interbrachial spaces, which, below, are nearly naked, as are the radial shields, while the remainder of the upper disk is densely covered with short, minute stumps, each bearing a crown of three or four thorns, or, rarely, a fork of two longer thorns. Radial shields long triangular, just touching without, diverging gently inward ; length to breadth 2.7 : 1.7. Seven, short, blunt, much flattened arm-spines, bearing

strong thorns on their edges; the second one is longest, and those below grow gradually shorter; lengths to that of an under arm-plate, 2.3, 3.5, 2.5, 2.2, 1.7, 1.5, .7 : .7. One minute tentacle-scale. Color in alcohol, above, disk pale greenish gray, arms of a faint pink.

Station 192, 129 fathoms, 1 specimen. Station, Cebu, Philippines, 95 – 100 fathoms, 1 specimen.

Ophiothrix cæspitosa sp. nov.

Plate XV. Figs. 417 - 420.

Special Marks. — Nine short, stout, much flattened, strongly toothed arm-spines. The puffed disk and small radial shields are set with short spines. Upper arm-plates transverse diamond-shaped, with lateral angles sharp.

Description of an Individual (Port Jackson). — Diameter of disk 7 mm. Length of arm 28 mm. Width of arm near disk 1.5. The vertical oval has about sixteen stout, blunt, nearly equal tooth-papillæ, whereof four or five are on the median line, and nearly as large as those on the margin. Four rather thin, squarish teeth, with a cutting edge making an obtuse angle. Mouth-shields small, closely joined to surrounding parts, broader than long, of a transverse, rounded oval shape, having a curve without and a very blunt, obtuse angle within. Side mouth-shields narrow, wider without than within, where they meet. Under arm-plates with ill-marked outlines of a rude, transverse oval form, with a curve without, lateral sides a little indented and the inner side vaguely angular. Side arm-plates with a low spine ridge. Upper arm-plates much wider than long, transverse diamond-shape, with lateral angles sharp and the outer one rounded; length to breadth .5 : 1.1. Disk thick, and puffed in the interbrachial spaces, thickly set near the margin with short, stout, stump-like spines rough at ends and sides, the longest .5 mm. in length. Towards the centre the spines grow fewer, and the middle region has scarcely any, so that the rounded overlapping scaling is conspicuous; next the mouth-shields, also, there are no spines. Radial shields small and triangular, much obscured by the short spines. Nine short, translucent, rather stout, blunt, flattened arm-spines, bearing pretty strong thorns on their edges; lengths to that of an under arm-plate, .8, 1.5, 1.8, 1.7, 1.3, 1.1, .9, .7, .4 : .5. One minute tentacle-scale at angle of under and side arm-plates. Color in alcohol, above, disk faint greenish; arms banded with lighter and darker yellowish brown.

Station, Port Jackson, 2 – 10 fathoms, 3 specimens.

In its disk this species resembles O. *triglochis*, but the arm-spines are much flatter and more toothed, and the upper arm-plates of a different shape.

Ophiothrix violacea Müll. & Trosch.

Ophiothrix violacea. Syst. Asterid., p. 115.

Off Brazil, 7 – 20 fathoms, 12+ specimens. Station 36, off Bermuda, 32 fathoms, 3 specimens. Fernando Noronha (same species?), shallow water, 1 specimen.

Ophiothrix Lütkeni? Wyv. Thom.

Ophiothrix Lütkeni. Depths of the Sea, 1872, p. 100.

Station 75, 450 fathoms, 1 specimen (young).

Ophiothrix propinqua Lym.

Ophiothrix propinqua. Proc. Bost. Soc. Nat. Hist., VIII., 1861, p. 83.

Tongatabu, 18 fathoms, 3 specimens (red var.). Fiji, Levuka Reefs, 2 specimens.

Ophiothrix purpurea v. Martens.

Ophiothrix purpurea. Monatsber. Kön. Akad., 1867, p. 346.

Station 176, 1450 fathoms [error? Sta. 177, 63 fms. ?], 3 specimens. Banda, 1 specimen.

Ophiothrix nereidina Müll. & Trosch.

Ophiothrix nereidina. Systerid. Ast., p. 115.

Zamboanga, Philippine Isl., 10 fathoms, 4 specimens.

Ophiothrix stelligera Lym.

Ophiothrix stelligera. Bull. Mus. Comp. Zoöl., III. 10, p. 237.

Aug. 7, 1874, 6 specimens. Station 186, 8 fathoms, 1 specimen. Arafura Sea, 1 specimen (same species?). Zamboanga, 10 fathoms, 1 specimen.

Ophiothrix Suensonii Ltk.

Ophiothrix Suensonii. Vid. Meddel., 1856, p. 16.

Station 36, 32 fathoms, 2 specimens.

Ophiothrix pusilla Lym.

Ophiothrix pusilla. Bull. Mus. Comp. Zoöl., III. 10, p. 235.

Station 208, 18 fathoms, 3 specimens.

Ophiothrix longipeda Müll. & Trosch.

Ophiothrix longipeda. Syst. Asterid., p. 113.

Station 186, 8 fathoms, 2 specimens. Ternate Shore, 1 specimen. 7 Aug., 1874, 1 specimen. Station 188, 28 fathoms, 2 specimens. Tongatabu, 18 fathoms, 1 specimen (same species?). Amboyna, 100 fathoms, 10 specimens (same species?). Zamboanga, 10 fathoms, 1 specimen.

Ophiothrix galateæ? Ltk.

Ophiothrix galatea. Ophiurid. Nov. Descr., 1872, p. 108.

Tongatabu, 18 fathoms.

Ophiothrix striolata Grube.

Ophiothrix striolata. Verhandl. Schlesisch. Ges., 1867, Pt. III. p. 99.

Station 208, 18 fathoms, 1 specimen. Zamboanga, Philippines, 10 fathoms, 1 specimen.

Ophiothrix Martensi Lym.

Ophiothrix Martensi. Bull. Mus. Comp. Zoöl., III. 10, p. 234.

Aug. 7, 1874, 4 specimens.

Ophiothrix exigua Lym.

Ophiothrix exigua. Bull. Mus. Comp. Zoöl., III. 10, p. 236.

Station 188, 28 fathoms, 1 specimen. Station 208, 18 fathoms, 1 specimen.

Ophiothrix ciliaris? Müll. & Trosch.

Ophiothrix ciliaris. Syst. Asterid., p. 114. Lym. Bull. Mus. Comp. Zoöl., III. 10, p. 233, Pl. IV. figs. 29-32.

Cebu, 95-100 fathoms, 1 specimen.

Ophiothrix triglochis Müll. & Trosch.

Ophiothrix triglochis. Syst. Asterid., p. 114.

Simon Bay, 5-18 fathoms, 3 specimens.

OPHIOCHITON Lym.

Ophiochiton lentus sp. nov.

Plate XIV. Figs. 398-400.

Special Marks. — Three stout arm-spines. Under arm-plates thickened, but not forming a distinct ridge. Scaling of disk smooth and uniform.

Description of an Individual (Station 171). — Diameter of disk 13 mm. Width of arm close to disk 2.5 mm. There are eleven short, sharp, stout, close-set mouth-papillæ on each angle, the two outermost and the one at the apex being a little larger than the rest. Mouth-shields about as broad as long, of a rounded heart-shape. Side mouth-shields extremely narrow, bent, wider without than within, where they meet. Under arm-plates large, swollen but not ridged, wider without than within, with lateral sides re-enteringly curved. Side arm-plates short and stout, with a low thick spine-ridge. Upper arm-plates twice as broad as long, of a fan-shape, with inner angle truncated, or a diamond-shape with much rounded angles. Disk round, smooth, and flat, covered with small, pretty uniform, rounded, overlapping scales, 2 or 3 in the length of 1 mm. Radial shields small, twice as long as broad, with much rounded corners, separated their entire length by two large round scales; length to breadth 2 : 1. Interbrachial spaces below covered with scaling similar to but finer than that above. Genital openings long, extending from outer corners of mouth-shield, where there are a few

minute papillæ, to margin of disk. Three stout, blunt, cylindrical, tapering, nearly equal arm-spines, about as long as an arm-joint. Two round, flat, tentacle-scales on the side arm-plate, whereof the one next the under arm-plate is much the smaller. Color in alcohol, pale gray.

Station 171, 600 fathoms, 1 specimen.

OPHIOGLYPHA Lym.

Ophioglypha meridionalis sp. nov.

Plate XVI. Figs. 447 - 449.

Special Marks. — Disk rather flat, covered with large imbricated scales. Arm-comb of minute bead-like papillæ, scarcely to be seen above, but continuous along edge of genital scale. Three peg-like arm-spines less than half as long as a joint. Only one tentacle-scale beyond the mouth-tentacles.

Description of an Individual (Station 320). — Diameter of disk 4 mm. Length of arm about 12 mm. Width of arm close to the disk .7 mm. Five small, short, broad, flat, close-set mouth-papillæ on each side of the mouth-angle, and one pointed and similar to the teeth at the apex. Mouth-shields somewhat swollen, about as broad as long, with a curve without and an obtuse angle inward. Side mouth-shields short, straight, meeting by their full width within, occupying only the inner angle of mouth-shield. First under arm-plate blunt heart-shaped, quite as large as, or larger than, the second, which is pentagonal, with inner angle truncated, outer side gently curved, and laterals re-enteringly curved ; one third out on the arm the under plates are small, much wider than long, bounded by a broad curve without and with a little peak inward. Side arm-plates large and thick, meeting broadly below beyond the second arm-plate, and touching above beyond the third plate. Upper arm-plates long wedge-shaped, with a clean curve outward and a sharp angle within. Disk rounded, rather flat and only a little arched above, covered by large slightly swollen scales, whereof the primary plates form a conspicuous rosette, radiating from which there usually is, in each interbrachial space, a row of three overlapping scales. Radial-shields as broad as long, sunken, rounded, with a faint angle inward ; joined without, separated by a wedge-scale within ; they are smaller than the large disk-scales. Below, the scales are similar, eight or nine in each interbrachial space. Papillæ along edge of genital scale minute, bead-like, and continuous ; only one or two, and sometimes none, can be seen from the upper surface. Three small, nearly equal, peg-like arm-spines, less than half the length of a side arm-plate. Five small, close-set tentacle-scales to pores of mouth-tentacles, three on one side and two on the other ; the pores beyond have but one small, rounded scale. Color in alcohol, straw.

Station 320, 600 fathoms, 1 specimen.

The single specimen, though well characterized, was perhaps not fully grown. It is the southern cousin of *O. robusta*, from which it differs in shorter arm-spines, more swollen disk-scales, smaller mouth-papillæ, and fewer tentacle-scales.

OPHIACANTHA Müll. & Trosch.

Ophiacantha discoidea sp. nov.

Plate XV. Figs. 405-407.

Special Marks. — Seven or eight slender, translucent, nearly smooth arm-spines. A small spine-like tentacle-scale. Disk densely set with minute stumps crowned with thorns.

Description of an Individual (Station 190). — Diameter of disk, 4.7 mm. Arms broken; they were plainly long, because, in their first 15 mm. there was scarcely any tapering. Width of arm near disk 1 mm. Three cylindrical, blunt, peg-like mouth-papillæ on each side, and a similar but longer one at apex of mouth-angle. Teeth longer than wide, with a rounded cutting edge. Mouth-shields broader than long, regular heart-shaped, with point inwards; length to breadth, .7 : 1. Side mouth-shields very wide without, and overlapping the first under arm-plate, but tapering to a thin point within, where they scarcely meet. First under arm-plate longer than broad, and somewhat overlapped by side mouth-shields; the plates just beyond are much wider than long, of a wide axe-shape, with a broad curve without, short re-entering curves on the sides, and an obtuse angle within. Side arm-plates meeting above and below, stout and flaring, with a strong spine-crest. Upper arm-plates fan-shaped, with the angle inward; widely separated. Disk nearly round, a little puffed, closely and evenly set, except in the middle, with very short microscopic stumps crowned with 3 or 4 little thorns. No scales or radial shields appear in the alcoholic specimen. Seven or eight slender, pointed, translucent, nearly smooth arm-spines, whereof the two uppermost are nearly as long as two joints; while those below gradually diminish in length to the lowest, which is two thirds as long as a joint. One narrow, pointed tentacle-scale. Color in alcohol, pale brownish gray.

Station 190, 49 fathoms, 1 specimen.

This species stands nearest, perhaps, to *O. cosmica*, from which it is distinguished by different under arm-plates, smaller side mouth-shields, stouter disk-stumps, and a very narrow spine-like tentacle-scale.

Ophiacantha Valenciennesi sp. nov.

Plate XV. Figs. 408-410.

Special Marks. — Disk evenly granulated above. Seven long, slender, much flattened arm-spines. Outer mouth-papilla spatula-like and covering the pore of the mouth-tentacle.

Description of an Individual (Station 192). — Diameter of disk 11 mm. Length of arm 50 mm. Width of arm near disk 3 mm. Twelve mouth-papillæ to each angle; of these the outermost one on either side is wide, like a short spatula, and is plainly the scale of the mouth-tentacle; the next four papillæ

are sharp and peg-like, the pair at apex of angle are thickened and conical. Five flat teeth, a little longer than wide, with a curved cutting edge. Mouth-shields long heart-shaped, or broad spear-head shaped; length to breadth 1.5 : 1.2. Side mouth-shields large and three-sided, wide without, tapering inward, where they nearly or quite meet. First under arm-plate small and wider than long; plates beyond, wide pentagonal, with outer side gently curved, laterals re-enteringly curved, and inner angle so obtuse and rounded as to be almost a gentle curve. Side arm-plates barely meeting below, separated above, rising in a thick abrupt spine-ridge. Upper arm-plates small, thick, and fan-shaped, with the angle inward. Disk thick and puffed, covered above by an even granulation, 9 or 10 grains in the length of 1 mm. On removing these, there is disclosed a smooth coat of very thin scales, about 5 in the length of 1 mm., which cover the radial shields, except their outer ends; interbrachial spaces below without grains, and covered with scales still finer than those above. Seven slender, much flattened arm-spines, slightly rough on the edges; the uppermost one extremely long, sometimes equal to five arm-joints, diminishing to the lowest, which is longer than one joint. Two large, oblong, slightly pointed tentacle-scales. Color in alcohol, pale brown above, much lighter below.

Station 192, 129 fathoms, 1 specimen.

Ophiacantha Normani sp. nov.

Plate XV. Figs. 414-416.

Special Marks. — Disk distinctly scaled and sparsely granulated, and with small, separated radial shields. A single row of grains along the outer edge of the basal upper arm-plates. Four smooth, slender spines, the upper ones longest.

Description of an Individual (Station 232). — Diameter of disk 12.5 mm. Length of arm about 40 mm. Width of arm next disk 2.5 mm. Seven widely spaced, cylindrical, tapering, peg-like mouth-papillæ, three on each side, and one at apex of mouth-angle. Mouth-shields a little broader than long, thick and square, with a little peak without and within; length to breadth 1 : 2. Side mouth-shields long and narrow, their outer end wedged between the first and second under arm-plates; not quite meeting within. First under arm-plate well marked, of a rounded triangular shape, with the point outward; third plate, and those just beyond it, broader than long, bounded without by a curve, on the sides by re-entering curves, and within by an angle; length to breadth (4th plate) 1.3 : 1.7. Side arm-plates with a swollen spine-ridge, meeting below, but separated above, stout, and, like the under plates, microscopically tuberculous. Upper arm-plates about as broad as long, short wedge-shaped, with outer side curved and a blunt angle within; the first three or four have, along their outer margin, a single row of rounded grains. Disk flat, somewhat angular, covered with well marked, pretty equal, overlapping scales, whose surface is sparsely set with rounded grains, similar to those of the upper arm-plates; interbrachial spaces below similarly covered, except that the scales are smaller

and obscured by skin. Radial shields small, ovoid, as long as broad, widely separated by a wedge of scales ; length to breadth 1.7 : 1.3. Genital openings wide, and extending quite from the mouth-shield to the disk margin. Four smooth, cylindrical, rather slender, blunt, tapering arm-spines, whereof the lowest is as long as an arm-joint, the two upper ones as long as a joint and a half, and the third intermediate. One rather large oval tentacle-scale. Color in alcohol, gray, with arm inclining to straw.

Station 232, 345 fathoms, 12+ specimens. Station 235, 565 fathoms, 1 specimen.

Ophiacantha abnormis sp. nov.

Plate XV. Figs. 411-413.

Special Marks. — Mouth-angles elongated, bearing, toward the apex, 12 or 14 slender, pointed papillæ. Six long, smooth, slender arm-spines. Disk sparsely set with very short spines.

Description of an Individual (Station 207). — Diameter of disk 11 mm. Length of arm, which is very attenuated near its end, 73 mm. Width of arm close to disk, without spines, 2.5 mm. Mouth-angles elongated, having no papillæ on their outer part near the mouth-tentacles, but on their inner portion bearing 4 or 5 slender, spaced papillæ on each side, and a cluster of 3 or 4 at the apex. Teeth wide and large, with a broad cutting edge. Mouth-shields broad triangular, with a small peak on the outer edge, and blunt angle within. Side mouth-shields short and extremely narrow, just meeting within. Under arm-plates thin and sunken, pentagonal, with a broad angle inward, outer edge straight, and deep re-entering curves on the lateral sides. Beyond the third, they are separated by the side arm-plates, which meet below and above and have a high, wide spine-ridge. Upper arm-plates triangular, somewhat swollen, with an angle inward, sharp lateral corners, and broad nearly straight outer edge, which on the basal plates bears two minute spines. Disk flat, having re-entering curves in the interbrachial spaces, and rather sparsely set with minute, short, blunt spines, which are fewer below. The outer ends of radial shields are exposed over the base of each arm. Genital openings long and large, extending from mouth-shield to disk margin. Six long, slender, smooth, cylindrical, tapering arm-spines, of which the two upper ones are as long as two arm-joints, thence diminishing in length to the lowest, which is about as long as half a joint. Pores large and tentacles very long ; on basal ones are two scales, of a pointed oval shape ; on those beyond, only one. Color in alcohol, straw.

Station 207, 700 fathoms, 12+ specimens. Station 210, 375 fathoms, 5 specimens.

In its elongated mouth-angles, this species somewhat resembles *O. hirsuta*, but its arm-spines are smooth and in all ways different.

NOTE ON THE STRUCTURE OF ASTROPHYTIDÆ.

In very early youth the Astrophytons bear a close resemblance to true Ophiurans, but they rapidly change with growth. Their structure will be more fully treated in the main work, and only two or three points of difference will here be suggested.

First, as to the arm covering. The young tip of an Astrophyton twig has the side arm-plates quite encircling it (Fig. 495), just as in an Ophiuran ; but already at the base of the same twig this plate is quite subordinate (Fig. 494 *i*), while at the base of the arm (Fig. 493 *i*) it occupies only the under surface, while the arm has risen in a high arch above it. It is not otherwise in the simple-armed *Astroschema* (Fig. 491 *i*). The upper arm-plates have no regular form, or stated mode of division ; but doubtless they are represented by a casing of very irregular scale-like pieces, to be found on the terminal branches of *Astrophyton*, and in the narrow belts of broken plates found in *Astroschema* (Fig. 491). The under arm-plates are extremely variable ; in the type of *Euryale asperum* they are essentially in one piece, and are constant to the end of the branches (Fig. 499 *h*), while for the type of *Astrophyton costosum* they are quite wanting, except perhaps the first one (Fig. 497 *h*), and are replaced by the large side arm-plates (Fig. 497 *i*) ; in the cold-water Astrophytons, such as *A. Agassizii*, they are plainly distinguished in the young, though divided in three pieces (Fig. 492 *h*, *h*). To such a structure of arm-plates the nearest approach among Ophiurans would perhaps be *Ophiomyxa.*

Secondly, as to the arm-spines. There are found, at the extreme tip of a twig of *Astrophyton* (Fig. 495), little hooklets on the side arm-plates ; when the arm has risen above the plate, and become quite distinct from it, there are found two or more large hooks (Fig. 494 *q*), which are the homologues of tentacle-scales, and which, nearer the base of the arm, usually become blunt spines (Fig. 493 *q*). In addition to these there are found on the twigs, in the true Astrophytons, *Astroclon*, *Astrocnida*, and among the simple-armed, in *Astrogomphus*, *Astroporpa*, *Astrochele,* and *Astrotoma*, two zones or belts of raised grains, each grain bearing a hooklet (Fig. 494). These belts of hook-bearing grains are therefore characteristic of a group among *Astrophytidæ;* while another is destitute of them, as *Euryale asperum* (Figs. 500, 501), *Trichaster*, *Astroceras*, *Astroschema* (Fig. 491), *Ophiocreas*, and *Astronyx.*

Thirdly, the mouth-shields among Astrophytons are quite subordinate, although so important among Ophiurans. Frequently there is but one (Fig. 492 *a*), and the position is very variable. The side mouth-shields, on the contrary, are usually very prominent (Figs. 492, 497, 499 *b*) ; so large are they in *Trichaster* that Müller and Troschel mistook them for a mouth-shield cut in two. The entirely different structure of *Euryale asperum* as exhibited in the figure (499), and especially the elongated side arm-plates (Fig. 501 *i*), absence of hook-bearing grains, and distinct build of mouth and under arm-plates, makes it advisable to remove the species from *Astrophyton* and restore to it the name *Euryale.* It is a question, also, whether the tropical Astrophytons

should not be generically distinguished. I have already shown, in considering those of the Hassler Expedition, the very different character of the arms (Figs. 496, 498), and the arrangement of their underlying hard parts is certainly quite different in the two (Figs. 492, 497).

ASTROTOMA Lym.

Astrotoma Murrayi sp. nov.

Plate XVIII. Figs. 474-476.

Special Marks. — Large tubercles, or smooth warts, on upper side of disk. No hooklets on belts of grains on arms, except close to their tip. Clusters of grains in interbrachial spaces next mouth.

Description of an Individual (Station 194). — Diameter of disk 29 mm. Length of arm 280 mm. Width of arm near disk 7 mm. Height of arm near disk 7 mm Apex of mouth-angle, embracing all the region of the jaw-plate, densely set with short, sharp, nearly equal, spine-like papillæ, thirty or more in number, and arranged in transverse rows of three or four. Lower surface and a part of the sides of the protuberant mouth-angles closely set with rounded and sometimes elongated grains. One round madreporic mouth-shield, 1.5 mm. in diameter, lying on the margin of the horizontal mouth-region, where it is separated from the vertical interbrachial space by a fold of skin stretched between the bases of the arms. Arms high, and tapering gradually to their tips, covered above and on the sides by belts of granules alternately raised and sunken. In the former the granules are larger and more distinct, and are more or less regularly arranged in four rows, whereof two at tip of arm bear minute, simple hooks, which, however, are soon rubbed off. In the latter, the granules are minute and arranged as a smooth pavement, in which appear many oblong holes or depressions. On its under surface the arm is covered by a cross-wrinkled, calcified skin, on which are scattered granules. Disk flat and angular, with re-entering curves in the interbrachial spaces; the radial shields, whose outlines are vaguely defined, are broad, and run nearly or quite to the centre. The upper surface is covered by a smooth pavement of small, soldered grains, among which appear small oblong depressions, and on whose surface are scattered a few large, smooth tubercles. The interbrachial spaces below are covered by a clump of large, coarse grains; at the inner end of each of these spaces is a deep, transverse hollow, at either extremity of which is a short, genital opening. Between the mouth-slit and lower margin of disk there are no tentacle-scales; but, beyond, each pore has four, rarely five, stout, smooth, peg-like scales, lying side by side, and nearly as long as an arm-joint; nearer tip of arm there are but three. Color in alcohol, reddish brown, the disk tubercles and clumps of grains about mouth being darker.

Station 194, 200 fathoms, 1 specimen.

ASTROCERAS * gen. nov.

Disk and arms covered with smooth, soft skin. Disk small; its interbrachial outlines re-enteringly curved; radial shields narrow and rather high, running nearly to centre. Arms somewhat knotted by a contraction between each pair of joints. Upper arm-plates divided in halves like high ribs, bearing a jointed spine at their upper end. Side arm-plates, towards middle of arm, having a long process to which are articulated the two spine-like tentacle-scales. Teeth. A clump of grains on sides of mouth-angles, answering to mouth-papillæ. Two vertical genital openings.

Astroceras stands next *Ophiocreas* and *Astroschema.* By its peculiar elongated side arm-plates bearing spine-like, rough tentacle-scales, and the large spines on the upper surface of the arm, it resembles the branching *Euryale asperum.*

Astroceras pergamena sp. nov.

Plate XVIII. Figs. 478 - 480.

Special Marks. — The smooth skin is translucent, allowing the underlying parts to be seen. The upper ends of the halves of the upper arm-plates project, and bear a stout spine. Tentacle-scales thick, rough ended, and nearly equal in size. On the sides of the mouth-angle are elongated grains answering to mouth-papillæ.

Description of an Individual (Station 235). — Diameter of disk 19 mm. Length of arm about 100 mm. Width of arm at base 2 mm.; height of same 2.5 mm. High up on the sides of the mouth-angles are elongated grains, irregularly arranged and answering to mouth-papillæ, while at the apex is the lowest tooth, flat and shaped like a wide spear-head. Mouth-shields very small, triangular, with a rounded angle inward and outer edge straight. Side mouth-shields very large and swollen, narrower without, meeting broadly within; both they and the mouth-shields are obscured by skin. Under arm-plates small, and squarish, and occupying only a part of the length of a joint. Side arm-plates nearly or quite meeting below, swollen and rounded, with a small projection to carry the two spine-like tentacle-scales; further out, on the arm, this projection is much elongated, forming an articulating process. Upper arm-plates represented by two rib-like ridges, which do not meet above, but project over the upper level of the arm, and bear a large, club-like, rough spine about 1.2 mm. long. Disk thin, and with deep constrictions in the interbrachial spaces. The smooth translucent skin allows the long and narrow radial shields to be seen; they are pointed within where they do not meet, and are separated their entire length; at their outer end they are elevated and carry a jointed spine, similar to that of the arms. The first pair of arm-pores has no tentacle-scales; but those beyond have two, which are thick and club-shaped, with

* ἀστήρ, star; κέρας, horn.

rough ends, and, unlike those of *Astroschema*, are nearly equal in size, and not much elongated towards the middle of the arm, where they bear bunches of minute hooks on their ends, and have a pedunculated look, owing to the elongation of the side arm-plates. Color in alcohol, light yellowish brown.

Station 235, 565 fathoms, 1 specimen.

OPHIOCREAS Lym.

In *Ophiocreas* and *Astroschema* the mouth gives almost no specific indications. It is by the character of the skin, or by the nature of its granulation, the thickness and length of the arms, their comparative height and breadth, and the form of the tentacle-scales and of the radial shields, that we get good specific marks.

Ophiocreas carnosus.

Plate XVI. Figs. 435 - 438.

Special Marks. — Animal covered by a smooth, soft, wrinkled skin. Tentacle-scales like rough-ended but not clubbed spines, which are short even at middle of the arm.

Description of an Individual * (Station 308). — Diameter of disk 15 mm. Length of arm 200 mm. Width of arm near disk 7 mm. ; height at the same point 6 mm. Mouth-angles so fleshy and puffed as to fill almost entirely the slits ; at the apex appears a small peg-like tooth ; upper teeth wider and spear-head shaped. On removing the thick, flabby skin, the usual large oblong side mouth-shields are seen, joined their entire length, except without, where they diverge somewhat to give place to the little mouth-shield. The side arm-plates are long, narrow, and curved, and meet fully below, separating the small, irregular, transversely oblong under arm-plates ; at their upper end they support the tentacle-scales, and unite with the belt of thin scales which represents the upper arm-plate. Disk thick, rising a little above the level of the arms, covered by a very thick, soft skin, which is especially wrinkled over the side mouth-shields. The same skin covers the arms, and is there loose and flabby. Radial shields narrow, rounded, thick and running quite to the centre. No tentacle-scale on first arm-pore ; the next five have one, in form of a small, blunt, thick spine enveloped in a sort of skin bag ; beyond, there are two, the lower of which, towards middle of arm, does not exceed 3 mm., and has a rough, but scarcely clubbed end. Color in alcohol, brownish pink, approaching flesh-color.

Station 308, 175 fathoms, 12+ specimens.

* The specimen described is not of the same size as the one figured.

Ophiocreas caudatus sp. nov.

Plate XVI. Figs. 439 - 442.

Special Marks. — A large species. Arms to disk as 13 to 1. No tentacle-scale on the first arm-joint; then for several joints only one, small and peg-like; thereafter two, which never grow very long. Skin thick.

Description of an Individual (Station 232). — Diameter of disk 22 mm. Length of arm about 300 mm. Width of arm close to disk 5.5 mm. Height of arm near base 5.5 mm. Mouth-angles covered with very thick skin giving a swollen look; on their sides and above the second mouth-tentacle is a sort of pavement of irregular flattened grains. Twelve large thick teeth, longer than wide, with cutting edge shaped like a rounded angle; the two lowest are smallest and are less flattened. Arm-joints obscurely indicated by the arm-bones, whose outlines are seen through the skin. Arms broader above than below; covered with a thick skin, which, when partly dry, presents under the microscope a minutely tuberculous surface. No tentacle-scale on first arm-joint; beyond this there is only one, short and peg-like, for some distance, sometimes as far as the thirteenth joint; after which there are two, still short, and cased in very thick bags of skin; on last third of arm the scale of the brachial side has become stout, thorny-ended, and much the longer (3 mm.). Disk thick and angular, covered with thick skin similar to that of the arms, and having interbrachial spaces re-enteringly curved. Radial shields high and narrow, diverging from the centre of disk to sides of the arms. The genital openings are long, extending from upper edge of disk to mouth-ring. Color in alcohol, uniform pinkish brown.

Station 232, off Enosima, 340 fathoms, 2 specimens.

Another somewhat smaller specimen had already two tentacle-scales on the fifth joint.

Ophiocreas abyssicola sp. nov.

Plate XVII. Figs. 470 - 473.

Special Marks. — Arms scarcely as high as wide, about eight times the diameter of the disk. Skin quite smooth, with radial shields scarcely indicated externally. Genital openings very short, and situated near the inner interbrachial angle.

Description of an Individual (Station 241). — Diameter of disk 7 mm. Length of arm about 60 mm. Width of arm close to disk 1.7 mm; height of same 1.2 mm. Four or five short, flat grains above the second mouth-tentacle, on the sides of each mouth-angle. Seven stout, nearly equal teeth, shaped like a blunt spear-head. On removing the skin the small, irregular, rounded mouth-shield, and large, longer than broad side mouth-shields, can be seen; the latter are often broken. Under arm-plates rather large, rounded, as broad as long, closely soldered, and with vague outlines. Side arm-plates small,

rounded, and swollen, closely joined with the under arm-plates. Arm-joints recognizable through the skin. Arms rounded and slender, tapering very gradually to the end. Disk flat and somewhat angular, not rising above level of arms, covered with soft, moderately thick skin. Radial shields shorter and wider than in other species, separated their entire length, and very thin and flat; from the outside they are scarcely indicated, and they do not meet in the centre. Two short, stout, bluntly pointed tentacle-scales, the lower one longer, and both nearly naked. Two very short genital openings, about 5 mm. long, near inner angle. When the skin is removed the genital plate and scale are seen, the plate being rounded, much longer than broad, tapering from without inward, and having the small, peg-like scale attached near its outer end. Color in alcohol, pale straw.

Station 241, 2,300 fathoms, 5 specimens.

This species, well distinguished from others, is remarkable for the great depth at which it lives. The genus is usually found not far below the 100-fathom line, and 500 fathoms may be considered deep for it.

Ophiocreas œdipus sp. nov.

Plate XVI. Figs. 443 - 446.

Special Marks. — Arms about twenty times the diameter of disk, and slender, except the base, which is swollen above, and contains the ovaries.

Description of an Individual (Station 344). — Diameter of disk 12 mm. Length of arm about 250 mm. Arm much swollen for the first four or five joints next disk, where its width is 3.5 mm., then suddenly shrinking to 2 mm. with a height of 2 mm. There are numerous small, flattened grains extending along the sides of the mouth-angles, above the second mouth-tentacle. Eight or nine broad, flat teeth, with well-rounded cutting edge, the two lowest being much narrower and peg-like. On removing the skin the mouth-shield is seen to be very small, a little longer than wide, with ends much rounded. Side mouth-shields very large, much longer than wide, with ends much rounded. Side mouth-shields very large, much longer than wide, somewhat swollen, meeting within where they are narrowest. Under arm-plates composed of two or more small pieces. Side arm-plates swollen, meeting below, and, at the base of the arm, joined to thick, narrow, ridge-like upper arm-plates, which arch upward, and nearly or quite meet on the median line. Disk angular and flat, with re-entering marginal curves. Radial shields narrow and highly arched, not quite meeting in the centre, covered with thin skin, which under the microscope is seen to be set with fine points. Genital openings large and wide, occupying the whole height of the disk. Where the skin is removed the genital plate is seen to be long, very broad and thick, tapering inward; the genital scale is small and peg-like. At base of arm there is only one tentacle-scale; beyond, there are two, the upper one very small, and spiniform, the lower one enclosed in a thick club-ended skin-bag.

On opening the singular swelling on the upper side of the base of the arm,

it is found to be a pouch full of large eggs, which are about .7 mm. long. In fact, the *ovaries* are in this species thus *pushed beyond the disk*, somewhat as in Star-fishes.

Color in alcohol, pinkish or yellowish brown.

Station 344, 420 fathoms, 3 specimens.

ASTROSCHEMA Ltk.

Astroschema horridum sp. nov.

Plate XVII. Figs. 458 - 461.

Special Marks. — Entire surface covered with little, swollen, oblong angular plates or scales, bearing minute points.

Description of a Specimen (Station 170). — Diameter of disk 12.5 mm. Length of arm 195 mm. Width of arm near disk 4.7 mm ; height of arm 4.2 mm. Seven stout, thickened, rather small teeth, of the usual short spear-head shape. The mouth-angles are paved with large, flattened, swollen grains, but have no true papillæ. Arms nearly cylindrical, very slightly swollen for their first 20 mm., beyond which they taper very regularly. They are evenly and pretty closely beset with minute points, like little blunt spines, about 4 in the length of 1 mm. ; these, on allowing the surface to dry, are seen to stand on small, swollen, oblong, angular plates or scales, which may be considered as exaggerated grains set with points. This covering continues quite to the end of the arm, where, however, the grains are more rounded and without points. Disk thick, rising a little above the arms, elegantly scalloped on its margin, with large radial shields (ribs), which are thick, swollen, and projecting at their outer ends, and taper inward to the centre, where they meet ; its surface is paved with little oblong, angular, swollen plates or scales, rather coarser than those of the arms, and bearing similar minute points. Genital openings straight, and occupying about one half the height of the disk. Mouth-tentacles enclosed in a tube of flat grains ; the next pair has no tentacle-scale ; the next one and those beyond have two, which are short at first, but about 40 mm. out become somewhat suddenly elongated, the upper one, about 1.3 mm. in length, remaining blunt spiniform, while the lower and larger takes on the form of a cylinder 3 mm. long, with a rough, swollen end. The two lines of pores lie closer together than usual, so that the furrow on the lower side of the arm is narrow. Color in alcohol, pale reddish brown.

Station 170, 630 fathoms, 1 specimen.

Astroschema salix sp. nov.

Plate XVII. Figs 466 - 469.

Special Marks. — Granulation fine, even, and close set; 7 or 8 grains in the length of 1 mm. Disk flat, with ill-distinguished radial shields. At tip of arm the lower tentacle-scale takes the form of a compound hook.

Description of an Individual (Station 170). — Diameter of disk 8.5 mm. Length of arm 85 mm. Width of arm near disk 3 mm. Height of arm 2.4 mm. Mouth-angles covered with minute, close, smooth granulation, and bearing at their apex the usual wide spear-head shaped teeth. Arms wide next disk, tapering rapidly for about 15 mm., and thence very gradually to their tips; covered by a fine, even, smooth, close-set granulation, 7 or 8 grains in the length of 1 mm. The skin, being thin, allows the outlines of the joints to show through, especially near the ends. Disk flat, scarcely rising above arms, and with a similar granulation, though rather looser on the upper surface. Radial shields scarcely to be distinguished, except at their outer ends. The first pair of pores outside mouth-slit has no scale; the next six have only one; those beyond two, whereof the inner and larger is cylindrical, with a somewhat swollen, rough end, and attains, about two thirds out on arm, a length of 1.3 mm. At the tip, the lower scale takes on the form of a flattened compound hook, with four curved teeth on its edge. Color in alcohol, very pale brown.

Station 170, 520 – 630 fathoms, 1 specimen.

Astroschema brachiatum sp. nov.

Plate XVII. Figs. 462 - 465.

Special Marks. — Arms twenty-four times the diameter of the disk, higher than wide, with a smooth, even granulation, 6 to 9 grains in the length of 1 mm.

Description of an Individual (Station 33). — Diameter of disk 11 mm. Length of arm 270 mm. Width of arm near disk 3 mm. Height of arm at same point 3.8 mm. The granulation of the disk is, as usual, projected over the mouth-angles, but there are no conspicuous grains which simulate mouth-papillæ. Teeth short, blunt, peg-like spines. Arms long, smooth, higher than wide, cleanly arched, and with only faint joint-ridges; they are closely and uniformly covered with a smooth granulation, 6 to 9 grains in the length of 1 mm. Disk high and arched, with well marked, somewhat elevated radial ribs, running nearly to the centre. The granulation is about as on the arms. Genital openings rather short; their upper ends not reaching the level of the top of the arm. No tentacle-scales (spines) on first pair of pores outside mouth-slit; the next two pairs have one scale, and those beyond two, of which the lower one attains a maximum length of 2 mm., and has a rough, slightly clubbed end. Color in alcohol uniform chocolate-brown.

Station 33, 435 fathoms, 1 specimen.

This species stands between *A. tenue* and *A. læve;* its arms are much thicker than those of the former, and much longer than those of the latter.

Astroschema tumidum sp. nov.

Plate XVII. Figs. 450 - 453.

Special Marks. — Disk and arms covered by regularly spaced, pointed, conical grains. The bases of the arms for two or three joints are strongly swollen.

Description of an Individual (Station 192). — Diameter of disk 8 mm. Length of arm 135 mm. Greatest width of arm, close to disk, 3.7 mm. Width, beyond the swelling, 2.3 mm. Height of arm, at same point, 1.8 mm. Seven or eight short, flat teeth, with a curved cutting edge ; the lowest one smallest. The general granulation of the disk is continued in a somewhat coarser form over the mouth-angles, and up their sides ; but there are no true mouth-papillæ. Arms well rounded, without any flattened surface, strongly swollen and ribbed, for the first two or three joints, but even and tapering beyond ; set with pointed conical grains which are regularly spaced, about 5 in the length of 1 mm., and which rarely touch each other. Disk strongly contracted in interbrachial spaces, and occupied chiefly by the high, wide radial shields (or ribs) which run quite to the centre ; granulation somewhat more sparse than on arms. On first arm-pore there is no tentacle ; the next has one, cylindrical, tapering and blunt, with sometimes a second rudimentary one ; the pores beyond have two, whereof the upper one is, as usual, much the smaller. One third out on the arm, the larger scale attains a length of 2 mm., and is rough at the end and slightly clubbed. Color in alcohol, pale yellowish brown, with interbrachial spaces of disk gray.

Station 192, 129 fathoms, 1 specimen.

This species presents the same swelled base of the arm found in *Ophiocreas œdipus*, and doubtless for the same purpose, an egg-pouch. The genera *Astroschema* and *Ophiocreas* though differing widely in their remote members, are, in their proximate species, only distinguished by surface granulation in the former.

Astroschema rubrum sp. nov.

Plate XVII. Figs. 454 - 457.

Special Marks. — Arms, at bases, not cleanly arched, but somewhat angular. Mouth-angles puffed so as to nearly close the slits. Granulation fine, smooth, and close-set, 6 or 7 in 1 mm. long. Tentacle scales short and scarcely club-ended.

Description of an Individual (Station 310). — Diameter of disk 12 mm. Length of arm 160 mm. Width of arm near disk, 3.5 mm. Height of arm 3.5 mm. Mouth-angles so swollen as nearly to close the slits, and covered by a smooth granulation much obscured by skin ; at the apex are small wide teeth. Arms near base as high as wide and not cleanly rounded, but inclined to be angular, and showing distinctly the outlines of arm-joints ; tapering uniformly ; near their ends higher than wide ; covered by a close-set, smooth, fine granulation, which, at bases of arms and on disk, has 6 or 7 grains in the length

of 1 mm. Disk thick, but flat on top, and rising but little above arms, covered by a thin skin, which is finely, closely and evenly granulated. The radial shields are faintly indicated by flat ridges running to the centre. Mouth-tentacles enclosed in tubes; the next have no scale; the next three or four have but one; those beyond, two, which at first are small and spiniform, and are nowhere long, the lower one attaining a maximum length of 1.4 mm. with a cylindrical form, and a rough scarcely swollen end. Color in alcohol, brownish red, approaching flesh-color.

Station 310, 400 fathoms, 4 specimens on a Gorgonian near *Brandellă.*

By its color and smooth surface *O. rubrum* may easily be mistaken for an *Ophiocreas.*

ASTROCLON * gen. nov.

Arms beginning to branch at a considerable distance from the disk, and having but few forks, nearly as in *Trichaster.* Disk rising well above the arms, and granulated, as are the latter. The tips of the twigs are encircled at each joint by a double belt of hook-bearing grains. Along the under surface of the base of the arm are two longitudinal lines of large, transverse slits, a pair to each joint, from which issue short tentacles; and above these on either side is a row of peg-like tentacle-scales. Mouth-angles naked on their sides, but with a bunch of spine-like papillæ at the apex. Two very large genital openings in each interbrachial space.

Astroclon propugnatoris † sp. nov.

Plate XVIII. Figs. 481-486.

Special Marks. — Animal covered above by a closely soldered granulation, in which appear numerous dark patches, which are small, oblong, smooth plates, sometimes raised like tubercles, and sometimes sunken. Below, the granulation is microscopic, and, on part of the under surface of arm, wanting. Five short, wide, smooth tentacle-scales.

Description of an Individual (Station 192). — Diameter of disk 65 mm. Length of arm: from disk to 1st fork, 160 mm.; from 1st fork to 2d, 36 mm.; 2d to 3d, 137 mm.; 3d to 4th, 26 mm.; 4th to 5th, 16 mm.; 5th to 6th, 16 mm.; 6th to end, 16 mm.; total, 407 mm. Width of arm near disk 14 mm.; height at same point 10 mm. Mouth-angles small, and on their sides smooth, bearing

* ἀστήρ, star; κλών, twig.

† Dr. Carpenter has happily translated "Challenger" by πρόμαχος, the Homeric word for a champion who stood in front of the line of battle and challenged the leaders of the enemy. *Propugnator* is a verbal translation, although it seems usually to signify rather a defender. I am told by high authority, however, that its present use is allowable. Goliath was such a challenging champion, but he is described in the Vulgate as *vir spurius,* an expression not applicable here.

at the apex a vertical tuft of small, smooth, short, spine-like papillæ. From near mouth to margin of disk the arms grow wider, but begin to taper from that point. They are cleanly arched above, but flat on the lower surface, a large portion of which is occupied by the deep, oblong, transverse pits (the largest 3.5 mm. long) on whose inner side stand the tentacles, so that this surface presents the appearance of a central, narrow, radiating strip, on whose sides are the tentacle-pits, arranged like the feathers of an arrow. This central strip has a very fine granulation, nearly obscured by skin; but the lateral region is quite smooth. The sides and upper surface are covered by a coat of soldered grains, about 2 in the length of 1 mm. Among them appear numerous small, smooth, slightly sunken, rounded, dark plates, usually 1.5 mm. in diameter; these begin near the tip, with a single plate on the upper surface of each joint, and gradually increase in number towards the base of the arm. The terminal twigs are encircled by double belts of hook-bearing grains (Fig. 486), but the intervening spaces are not yet granulated. Disk thick, rising well above arms; covered above by a soldered granulation similar to that of the arm, with scattered smooth plates, which sometimes are raised and sometimes sunken. Interbrachial spaces below covered by a minute granulation, which is more or less obscured by skin, and seems smooth to the naked eye. Radial shields not externally indicated. Genital openings very large, extending from opposite the second tentacle-pit nearly to margin of disk, and capable of great distention; one of them was open to the width of 9 mm. The mouth-tentacles and first pair on the arm have no tentacle-scales; thence to margin of disk there are two or three, minute and peg-like, to each tentacle; for some distance beyond the margin each tentacle has five small, thick, short, wide scales, about 1.5 mm. long, arranged in a single line. Color in alcohol, uniform yellowish brown, with chocolate patches where the smooth plates are.

Station 192, 129 fathoms, 1 specimen.

The single specimen had lost one arm and a piece of the disk, the result apparently of an injury, and not of self-division.

There was sent me recently a single Ophiuran of this Expedition, which has most singular arm-spines, like round-headed nails, or long-handled parasols. They are arranged, not in one, but in *several* rows, thus forming an exception to all other genera in the group. There is a similar species, but of quite a different genus, in the collection of the second "Blake" Expedition; and I propose to prepare on these a separate paper.

DESCRIPTION OF PLATES.

PLATE XI.

Fig. 278. *Amphiura maxima,* below ; $\frac{5}{2}$.
Fig. 279. " " above ; $\frac{5}{2}$.
Fig. 280. " " tentacle-scales ; $\frac{5}{1}$.
Fig. 281. " " arm-spines ; $\frac{5}{2}$.
Fig. 282. " *bellis,* below ; $\frac{7}{2}$.
Fig. 283. " " above ; $\frac{7}{2}$.
Fig. 284. " " arm-spines ; $\frac{7}{2}$.
Fig. 285. " *incana,* below ; $\frac{4}{1}$.
Fig. 286. " " radial shields ; $\frac{4}{1}$.
Fig. 287. " " arm-spines ; $\frac{4}{1}$.
Fig. 288. " *argentea,* below ; $\frac{6}{1}$.
Fig. 289. " " above ; $\frac{6}{1}$.
Fig. 290. " " arm-spines ; $\frac{6}{1}$.
Fig. 291. " " joints near tip ; $\frac{6}{1}$.
Fig. 292. " *acacia,* below ; $\frac{5}{1}$.
Fig. 293. " " above ; $\frac{5}{1}$.
Fig. 294. " " arm-spines ; $\frac{5}{1}$.
Fig. 295. " *constricta,* below ; $\frac{7}{1}$.
Fig. 296. " " above ; $\frac{7}{1}$.
Fig. 297. " " arm-spines ; $\frac{7}{1}$.
Fig. 298. " " joints near tip of arm ; $\frac{7}{1}$.
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Fig. 300. " " above ; $\frac{5}{1}$.
Fig. 301. " " arm-spines ; $\frac{5}{1}$.
Fig. 302. " *iris,* below ; $\frac{5}{1}$.
Fig. 303. " " above ; $\frac{5}{1}$.
Fig. 304. " " arm-spines ; $\frac{5}{1}$.
Fig. 305. " *lanceolata,* below ; $\frac{6}{1}$.
Fig. 306. " " above ; $\frac{6}{1}$.
Fig. 307. " " arm-spines ; $\frac{6}{1}$.
Fig. 308. " *glabra,* below ; $\frac{6}{1}$.
Fig. 309. " " above ; $\frac{6}{1}$.
Eig. 310. " " arm-spines ; $\frac{6}{1}$.
Fig. 311. " *angularis,* below ; $\frac{4}{1}$.
Fig. 312. " " above ; $\frac{4}{1}$.
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Fig. 314. " *dilatata,* below ; $\frac{6}{1}$.
Fig. 315. " " above ; $\frac{6}{1}$.
Fig. 316. " " arm-spines ; $\frac{6}{1}$.

PLATE XII.

Fig. 317. *Amphiura concolor*, below ; $\frac{4}{1}$.
Fig. 318. " " above ; $\frac{4}{1}$.
Fig. 319. " " arm-spines ; $\frac{4}{1}$.
Fig. 320. " *dalea*, below ; $\frac{7}{2}$.
Fig. 321. " " above ; $\frac{7}{2}$.
Fig. 322. " " arm-spines ; $\frac{7}{2}$.
Fig. 323. " *cernua*, below ; $\frac{6}{1}$.
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Fig. 325. " " arm-spines ; $\frac{6}{1}$.
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Fig. 328. " " arm-spines ; $\frac{6}{1}$.
Fig. 329. " *Verrilli*, below ; $\frac{6}{1}$.
Fig. 330. " " above ; $\frac{6}{1}$.
Fig. 331. " " arm-spines ; $\frac{6}{1}$.
Fig. 332. " *canescens*, below ; $\frac{6}{1}$.
Fig. 333. " " above ; $\frac{6}{1}$.
Fig. 334. " " arm-spines ; $\frac{6}{1}$.
Fig. 335. " *patula*, below ; $\frac{5}{2}$.
Fig. 336. " " above ; $\frac{5}{2}$.
Fig. 337. " " arm-joints ; $\frac{5}{2}$.
Fig. 338. *Amphilepis patens*, below ; $\frac{3}{1}$.
Fig. 339. " " above ; $\frac{3}{1}$.
Fig. 340. " " arm-spines ; $\frac{3}{1}$.
Fig. 341. *Ophiocnida pilosa*, below ; $\frac{7}{1}$.
Fig. 342. " " above ; $\frac{7}{1}$.
Fig. 343. " " arm-spines ; $\frac{7}{1}$.
Fig. 344. " *scabra*, below ; $\frac{6}{1}$.
Fig. 345. " " above ; $\frac{6}{1}$.
Fig. 346. " " arm-spines ; $\frac{6}{1}$.

PLATE XIII.

Fig. 347. *Ophiactis flexuosa*, below ; $\frac{4}{1}$.
Fig. 348. " " above ; $\frac{4}{1}$.
Fig. 349. " " arm-spines ; $\frac{4}{1}$.
Fig. 350. " *nama*, below ; $\frac{5}{1}$.
Fig. 351. " " above ; $\frac{5}{1}$.
Fig. 352. " " arm-spines ; $\frac{5}{1}$.
Fig. 353. " *canotia*, below ; $\frac{5}{1}$.
Fig. 354. " " above ; $\frac{5}{1}$.
Fig. 355. " " arm-spines ; $\frac{5}{1}$.
Fig. 356. " *poa*, below ; $\frac{6}{1}$.
Fig. 357. " " above ; $\frac{6}{1}$.
Fig. 358. " " arm-spines ; $\frac{6}{1}$.
Fig. 359. " *cuspidata*, below ; $\frac{6}{1}$.

Fig. 360. *Ophiactis cuspidata*, above ; $\frac{5}{1}$.
Fig. 361. " " arm-spines ; $\frac{5}{1}$.
Fig. 362. " *resiliens*, below ; $\frac{4}{1}$.
Fig. 363. " " above ; $\frac{4}{1}$.
Fig. 364. " " arm-spines ; $\frac{4}{1}$.
Fig. 365. " *hirta*, below ; $\frac{6}{1}$.
Fig. 366. " " above ; $\frac{6}{1}$.
Fig. 367. " " arm-spines ; $\frac{6}{1}$.
Fig. 368. *Ophiostigma africanum*, below ; $\frac{10}{1}$.
Fig. 369. " " above ; $\frac{10}{1}$.
Fig. 370. " " arm-spines ; $\frac{10}{1}$.
Fig. 371. *Ophiochondrus stelliger*, below ; $\frac{5}{1}$.
Fig. 372. " " above ; $\frac{5}{1}$.
Fig. 373. " " arm-spines ; $\frac{5}{1}$.
Fig. 374. *Ophiopholis japonica*, below ; $\frac{7}{2}$.
Fig. 375. " " above ; $\frac{7}{2}$.
Fig. 376. " " arm-joints, profile ; $\frac{7}{2}$.

PLATE XIV.

Fig. 377. *Ophioconis pulverulenta*, below ; $\frac{3}{1}$.
Fig. 378. " " above ; $\frac{3}{1}$.
Fig. 379. " " arm-spines ; $\frac{3}{1}$.
Fig. 380. " *antartica*, below ; $\frac{5}{2}$.
Fig. 381. " " above ; $\frac{5}{2}$.
Fig. 382. " " arm-joints, profile ; $\frac{5}{2}$.
Fig. 383. *Ophiomyces grandis*, below ; $\frac{4}{1}$.
Fig. 384. " " above ; $\frac{4}{1}$.
Fig. 385. " " arm-spines ; $\frac{4}{1}$.
Fig. 386. " *spathifer*, below ; $\frac{15}{2}$.
Fig. 387. " " above ; $\frac{15}{2}$.
Fig. 388. " " arm-spines ; $\frac{15}{2}$.
Fig. 388 A. " " mouth-angle ; $\frac{15}{1}$.
Fig. 389. *Pectinura heros*, below ; $\frac{3}{2}$.
Fig. 390. " " above ; $\frac{3}{2}$.
Fig. 391. " " arm-joint, profile ; $\frac{3}{2}$.
Fig. 392. " *arenosa*, below ; $\frac{7}{2}$.
Fig. 393. " " above ; $\frac{7}{2}$.
Fig. 394. " " arm-joint, profile ; $\frac{7}{2}$.
Fig. 395. *Ophiopeza aster*, below ; $\frac{5}{2}$.
Fig. 396. " " above ; $\frac{5}{2}$.
Fig. 397. " " arm-joints, profile ; $\frac{5}{2}$.
Fig. 398. *Ophiochiton lentus*, below ; $\frac{5}{2}$.
Fig. 399. " " above ; $\frac{5}{2}$.
Fig. 400. " " arm-joint, profile ; $\frac{5}{2}$.
Fig. 401. *Ophiothrix capillaris*, below ; $\frac{3}{2}$.
Fig. 402. " " above ; $\frac{3}{2}$.
Fig. 403. " " arm-joints, profile ; $\frac{3}{2}$
Fig. 404. " " spine ; $\frac{5}{2}$.

PLATE XV.

Fig. 405. *Ophiacantha discoidea*, below ; $\frac{9}{2}$.
Fig. 406. " " above ; $\frac{9}{2}$.
Fig. 407. " " arm-spines ; $\frac{9}{2}$.
Fig. 408. " *Valenciennesi*, below ; $\frac{3}{1}$.
Fig. 409. " " above ; $\frac{3}{1}$.
Fig. 410. " " arm-spines ; $\frac{3}{1}$. Some of these spines broken: they are really longer.
Fig. 411. *Ophiacantha abnormis*, below ; $\frac{2}{1}$.
Fig. 412. " " above ; $\frac{2}{1}$. Minute spines on outer edge of upper arm-plate omitted.
Fig. 413. *Ophiacantha abnormis*, arm-spines ; $\frac{2}{1}$.
Fig. 414. " *Normani*, below ; $\frac{5}{2}$.
Fig. 415. " " above ; $\frac{5}{2}$.
Fig. 416. " " arm-spines ; $\frac{5}{2}$.
Fig. 417. *Ophiothrix cæspitosa*, below ; $\frac{5}{1}$.
Fig. 418. " " above ; $\frac{5}{1}$.
Fig. 419. " " spine ; $\frac{15}{2}$.
Fig. 420. " " arm-joint, profile ; $\frac{5}{1}$.
Fig. 421. " *aristulata*, below ; $\frac{5}{2}$.
Fig. 422. " " above ; $\frac{5}{2}$.
Fig. 423. " " arm-joint, profile ; $\frac{5}{2}$.
Fig. 424. " " spine ; $\frac{4}{1}$.
Fig. 425. " *berberis*, below ; $\frac{3}{1}$.
Fig. 426. " " above ; $\frac{3}{1}$.
Fig. 427. " " spine ; $\frac{3}{2}$.
Fig. 428. " " arm-joint, profile ; $\frac{3}{1}$.

PLATE XVI.

Fig. 429. *Amphilepis papyracea*, below ; $\frac{7}{1}$.
Fig. 430. " " above ; $\frac{7}{1}$.
Fig. 431. " " arm-joints ; $\frac{7}{1}$.
Fig. 432. " *tenuis*, below ; $\frac{6}{1}$.
Fig. 433. " " above ; $\frac{6}{1}$.
Fig. 434. " " arm-joints ; $\frac{6}{1}$.
Fig. 435. *Ophiocreas carnosus*, below ; $\frac{1}{1}$.
Fig. 436. " " above ; $\frac{1}{1}$.
Fig. 437. " " arm-joint near base of arm ; $\frac{1}{1}$
Fig. 438. " " arm-joint near tip of arm ; $\frac{1}{1}$.
Fig. 439. " *caudatus*, below ; $\frac{1}{1}$.
Fig. 440. " " above ; $\frac{1}{1}$.
Fig. 441. " " arm-joint near base of arm ; $\frac{2}{1}$.
Fig. 442. " " arm-joint near tip of arm ; $\frac{2}{1}$.
Fig. 443. " *œdipus*, below ; $\frac{2}{1}$.
Fig. 444. " " above ; $\frac{2}{1}$.
Fig. 445. " " arm-joint near base of arm ; $\frac{2}{1}$.

Fig. 446. *Ophiocreas œdipus*, arm-joint near tip of arm ; $\frac{2}{1}$.
Fig. 447. *Ophioglypha meridionalis*, below ; $\frac{5}{1}$.
Fig. 448. " " above ; $\frac{6}{1}$.
Fig. 449. " " arm-joints ; $\frac{6}{1}$.

PLATE XVII.

Fig. 450. *Astroschema tumidum*, below ; $\frac{5}{2}$.
Fig. 451. " " above ; $\frac{5}{2}$.
Fig. 452. " " arm-joint near base of arm ; $\frac{5}{2}$.
Fig. 453. " " arm-joint near tip of arm ; $\frac{5}{2}$.
Fig. 454. " *rubrum*, below ; $\frac{2}{1}$.
Fig. 455. " " above ; $\frac{2}{1}$.
Fig. 456. " " arm-joint near base of arm ; $\frac{2}{1}$.
Fig. 457. " " arm-joint near tip of arm ; $\frac{2}{1}$.
Fig. 458. " *horridum*, below ; $\frac{2}{1}$.
Fig. 459. " " above ; $\frac{2}{1}$.
Fig. 460. " " near base of arm ; $\frac{2}{1}$.
Fig. 461. " " near tip of arm ; $\frac{2}{1}$.
Fig. 462. " *brachiatum*, below ; $\frac{2}{1}$.
Fig. 463. " " above ; $\frac{2}{1}$.
Fig. 464. " " near base of arm ; $\frac{2}{1}$.
Fig. 465. " " near tip of arm ; $\frac{2}{1}$.
Fig. 466. " *salix*, below. Grains on sides of mouth-angles too large ; $\frac{3}{1}$.
Fig. 467. " " above ; $\frac{3}{1}$.
Fig. 468. " " near base of arm ; $\frac{3}{1}$.
Fig. 469. " " near tip of arm ; $\frac{3}{1}$.
Fig. 470. *Ophiocreas abyssicola*, below ; $\frac{7}{2}$.
Fig. 471. " " above ; $\frac{7}{2}$.
Fig. 472. " " near base of arm ; $\frac{7}{2}$.
Fig. 473. " " near tip of arm ; $\frac{7}{2}$.

Plate XVIII.

Fig. 474. *Astrotoma Murrayi*, below ; $\frac{1}{1}$.
Fig. 475. " " above ; $\frac{1}{1}$.
Fig. 476. " " arm-joints ; $\frac{1}{1}$.
Fig. 477. *Astroceras pergamena*, below ; $\frac{5}{2}$.
Fig. 478. " " above ; $\frac{5}{2}$.
Fig. 479. " " near base of arm ; $\frac{5}{2}$.
Fig. 480. " " near tip of arm ; $\frac{5}{2}$.
Fig. 481. *Astroclon propugnatoris*, below ; $\frac{1}{1}$.
Fig. 482. " " above ; $\frac{1}{1}$.
Fig. 483. " " profile ; $\frac{1}{1}$.
Fig. 484. " " near base of arm ; $\frac{1}{1}$.
Fig. 485. " " beyond 1st fork ; $\frac{1}{1}$.
Fig. 486. " " tip of twig ; $\frac{5}{1}$.

Plate XIX.

a, mouth-shield ; *b*, side mouth-shield ; *c*, jaws ; *d*, mouth-papillæ ; *d''*, tooth-papillæ ; *e*, jaw-plate ; *h*, under arm-plate ; *i*, side arm-plate ; *n*, genital scale ; *o*, genital plate ; *q*, tentacle-scales ; *r*, tentacle ; *y*, outer articulating prominence of an arm-bone ; *β*, inner articulating prominence of an arm-bone.

Fig. 487. *Euryale asperum.* Outer face of an arm-bone ; *y*, articulating prominence. $\frac{2}{1}$.

Fig. 488. *E. asperum.* Inner face of next bone ; *β*, articulating prominence of the "hour-glass" shape. $\frac{2}{1}$.

Fig. 489. *E. asperum.* Widened outer face of an arm-bone at a fork ; *y*, new articulating prominence connecting with one new branch. $\frac{2}{1}$.

Fig. 490. *E. asperum.* Inner face of next bone, split nearly in two, and bearing two articulating prominences, *β*. $\frac{2}{1}$.

Fig. 491. *Astroschema oligactes.* A joint of the arm near its end, with the skin split to show the thick, squarish side arm-plate (*i*), with the broken pieces above, which answer to upper arm-plates ; the longer tentacle-scale, like a spine (*q*) ; and the tentacle (*r*). $\frac{5}{1}$.

Fig. 492. *Astrophyton Agassizii.* A portion of the mouth and under surface of the disk in a very young specimen. *a*, madreporic radial shield ; *b*, large side mouth-shield ; *c*, jaw ; *d''*, tooth-papillæ ; *e*, jaw-plate ; *h*, *h*, under arm-plate, divided in three pieces ; *i*, large side arm-plates, meeting below ; *n*, genital scale ; *o*, genital plate ; *q*, tentacle-scales or arm-spines. $\frac{10}{1}$.

Fig. 493. *A. Agassizii.* Arm-joint near base of arm, showing the side arm-plate (*i*) and the spine-like tentacle-scales (*q*). $\frac{4}{1}$.

Fig. 494. *A. Agassizii.* Joint of a twig near end of arm, in profile, to show the side arm-plate (*i*) and the hooked tentacle-scale (*q*). Above is the characteristic double belt of grains, each bearing a hook. $\frac{25}{1}$.

Fig. 495. *A. Agassizii.* Tip of a twig, showing the side arm-plates encircling the arm, and bearing little hooks. $\frac{37}{1}$.

Fig. 496. *Astrophyton Pourtalesii.* Portion of under surface of disk, showing the narrow arm characteristic of this section of the genus. $\frac{1}{2}$.

Fig. 497. *Astrophyton costosum.* A portion of mouth and under side of disk, with the skin removed to show the underlying hard parts ; lettered like Fig. 492. $\frac{2}{1}$.

Fig. 498. *Astrophyton spinosum.* Portion of under surface of disk, showing the wide arm characteristic of this section of the genus. $\frac{1}{2}$.

Fig. 499. *Euryale asperum.* A part of mouth and surrounding parts with the skin removed ; lettered as in Fig. 492. $\frac{2}{1}$.

Fig. 500. *E. asperum.* Joints near tip of arm, to show transition from hook-like tentacle-scales (*q*) to those of a stumpy shape. They are carried by the elongated side arm-plates (*i*). Above is seen a large dorsal spine. $\frac{25}{1}$.

Fig. 501. *E. asperum.* Joint close to tip of arm, in profile, to show the greatly elongated side arm-plate (*i*), bearing two hook-like tentacle-scales (*q*). It was this structure that Dr. Ludwig took for a pedicellaria.

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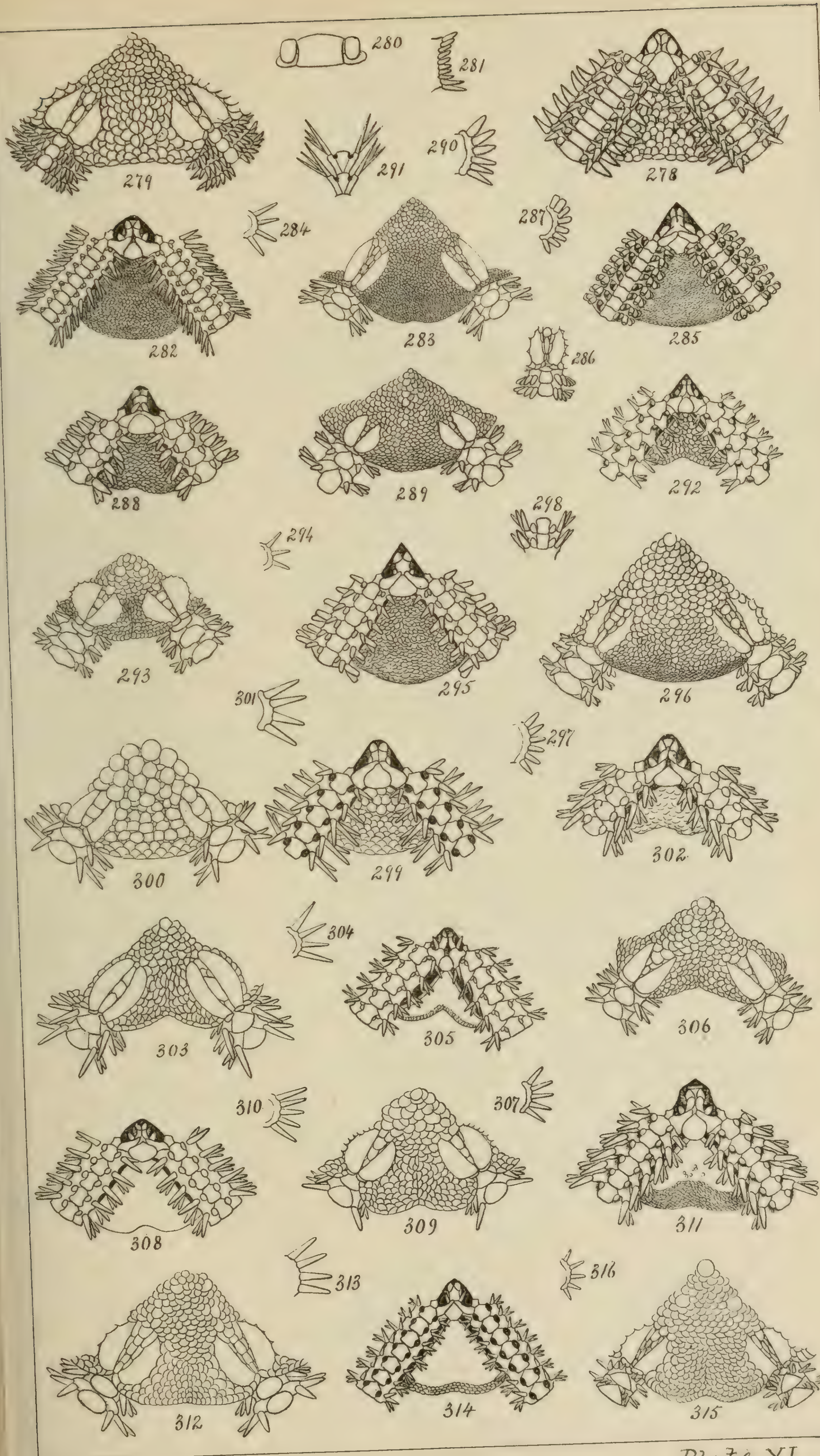

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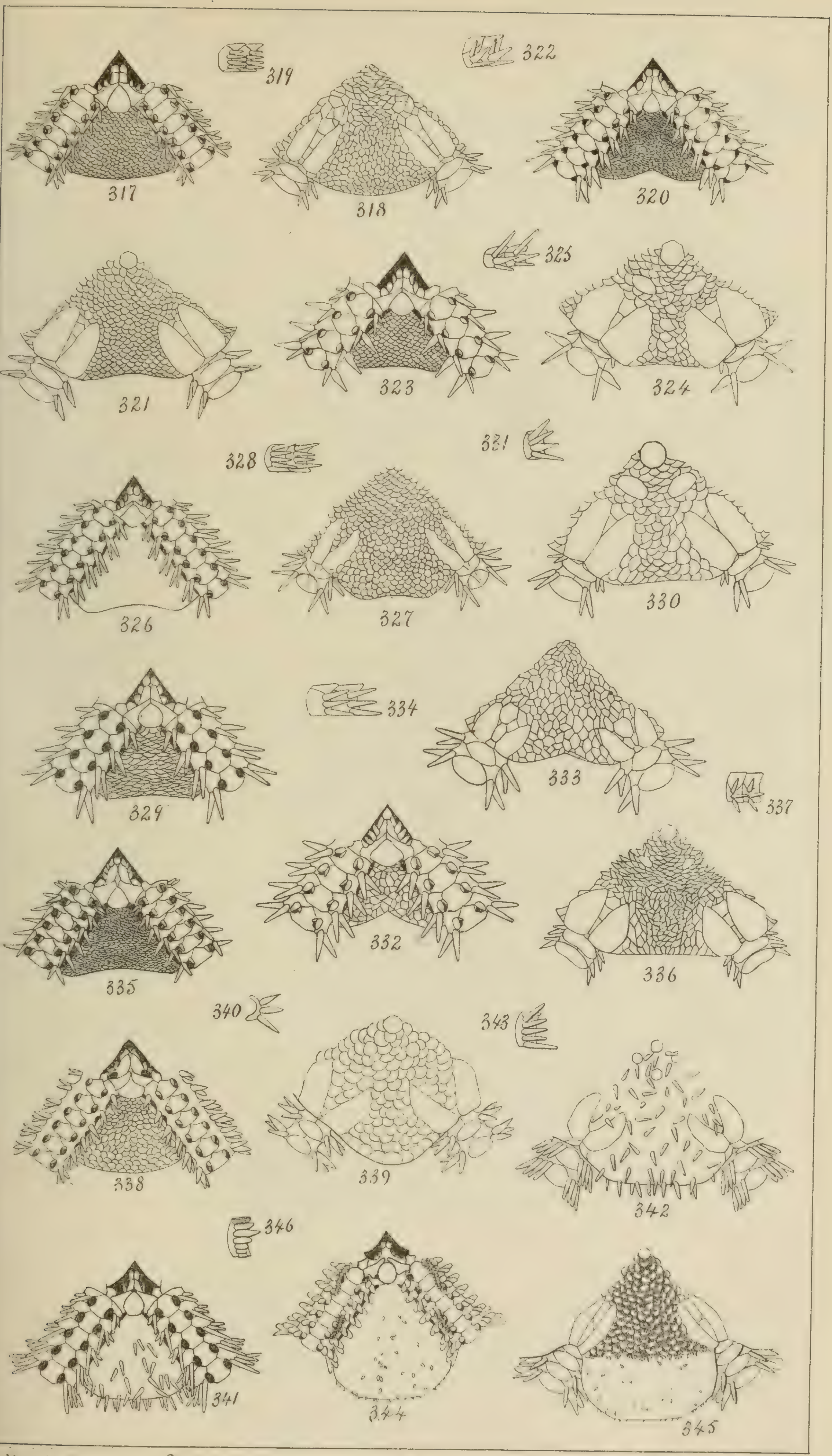

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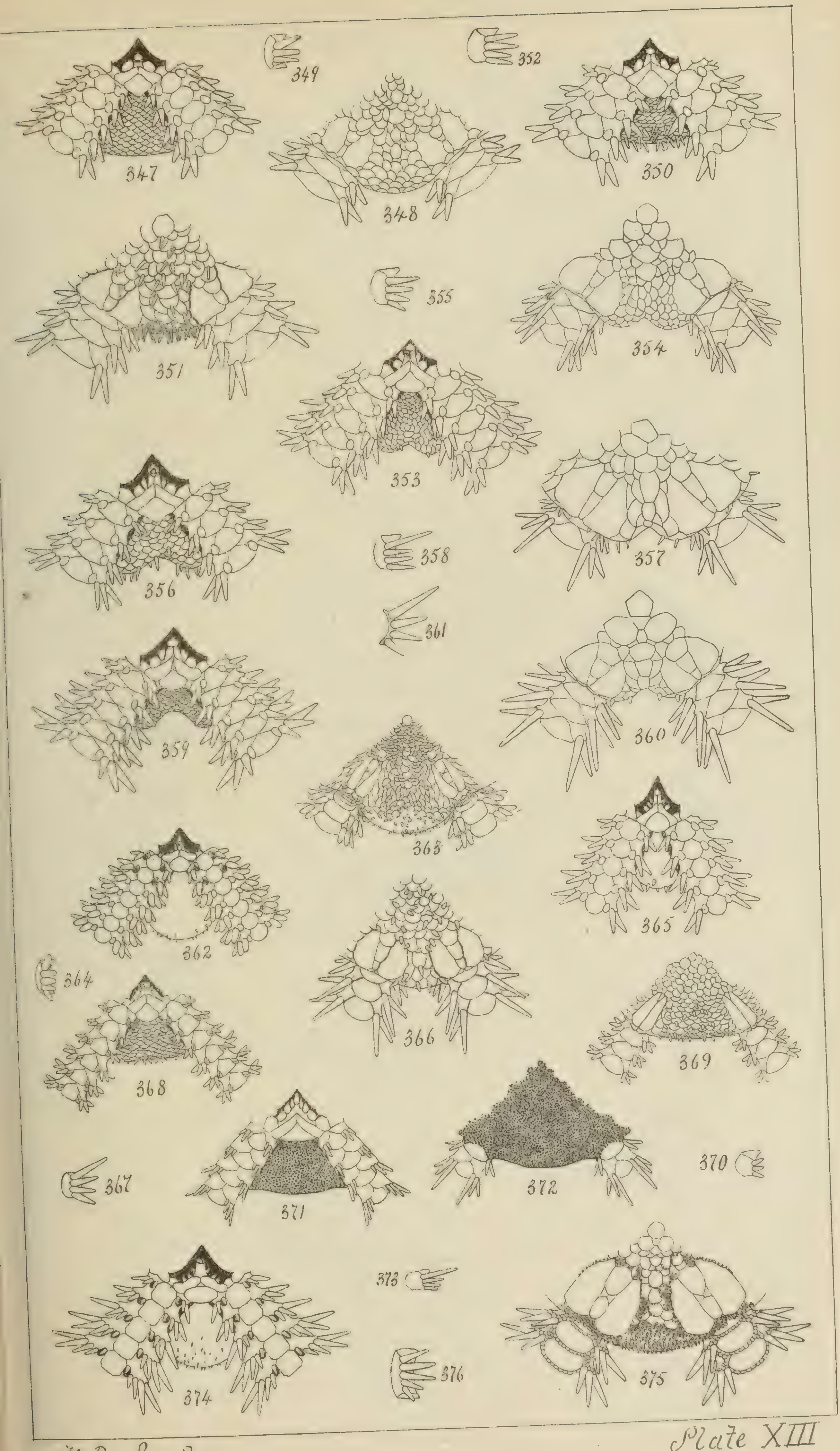
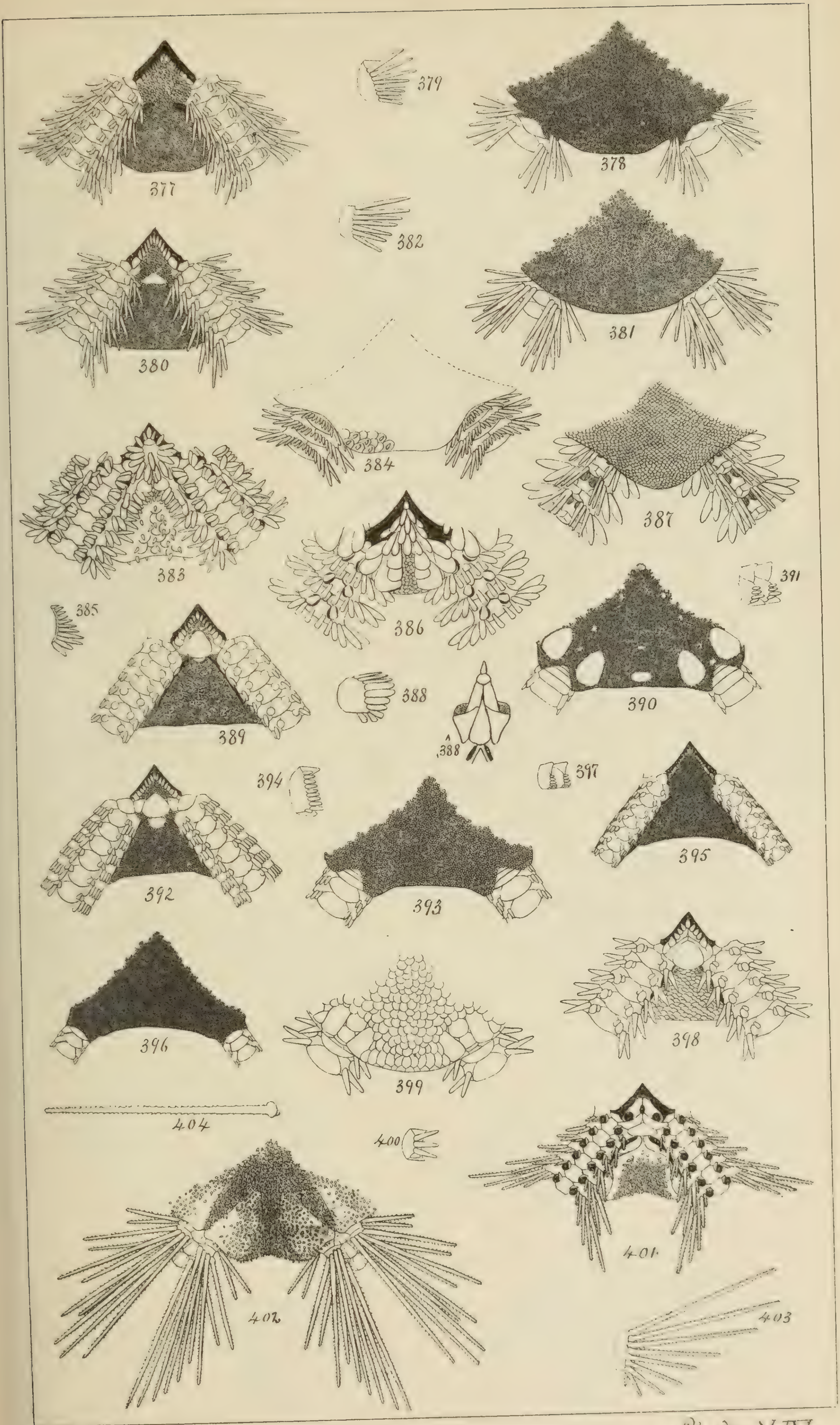


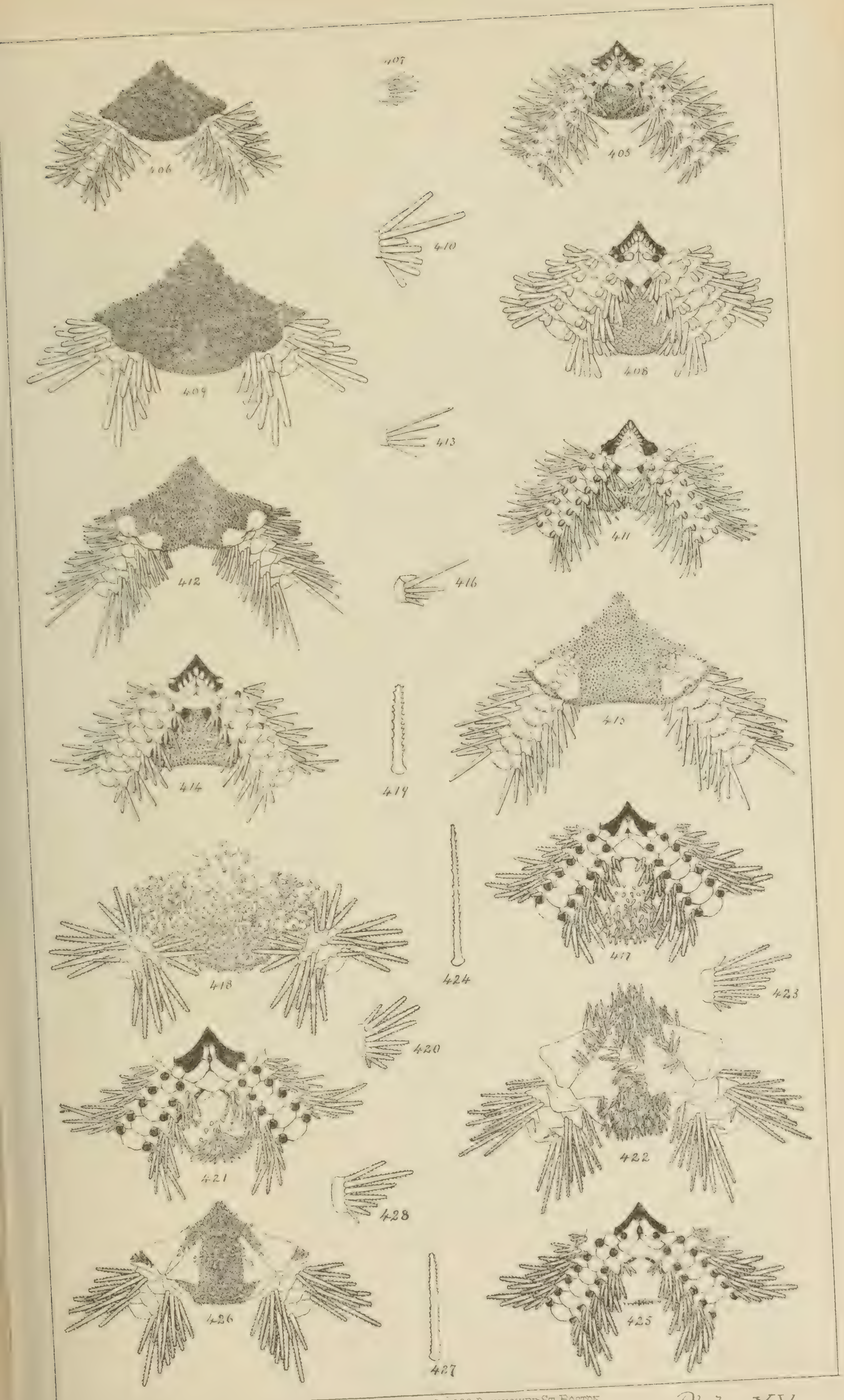
K. P. ad nat.

Plate XIII



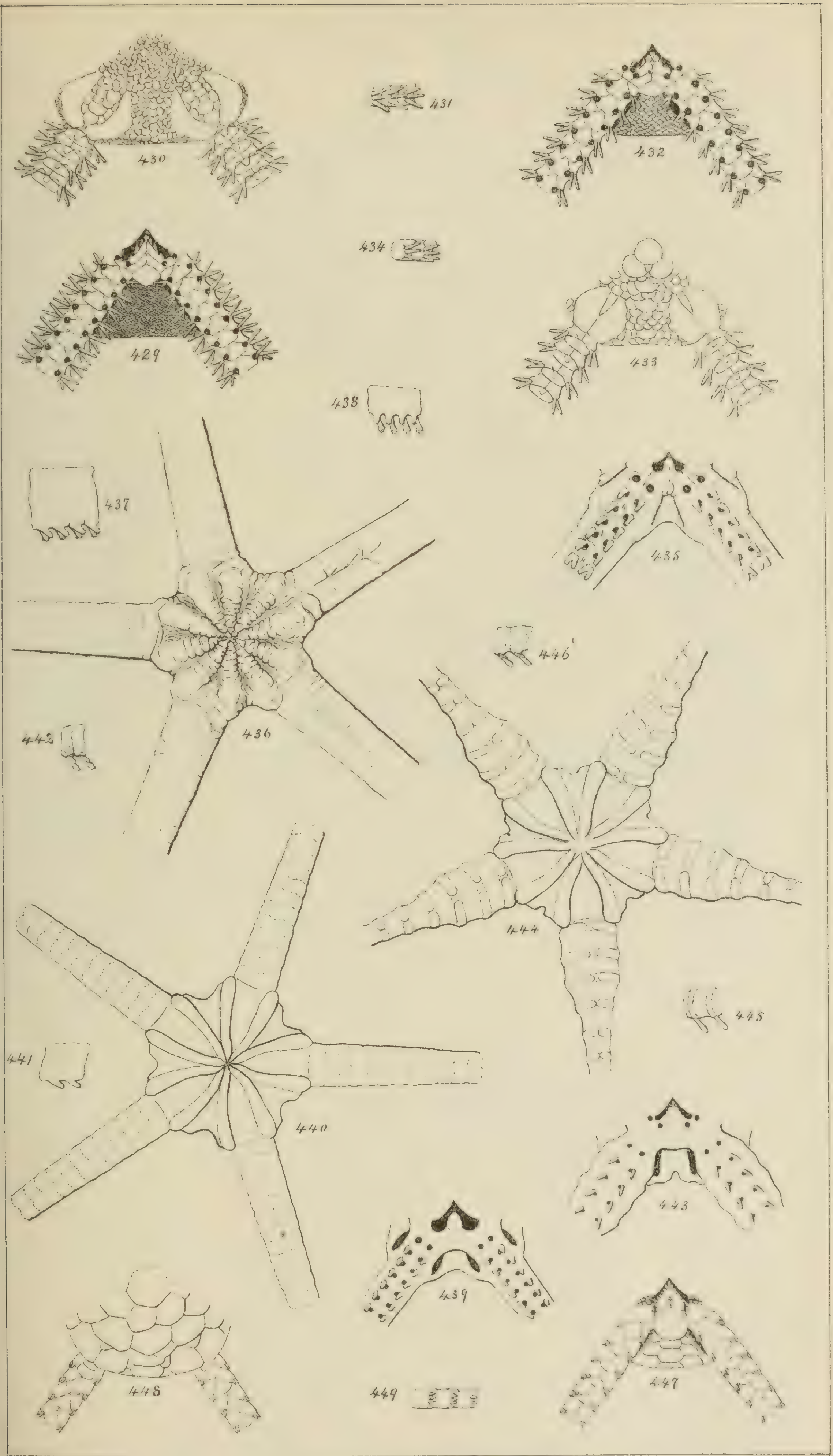
K.P. ad nat.

Plate XIV



THE HELIOTYPE PRINTING CO. 220 DEVONSHIRE ST. BOSTON.

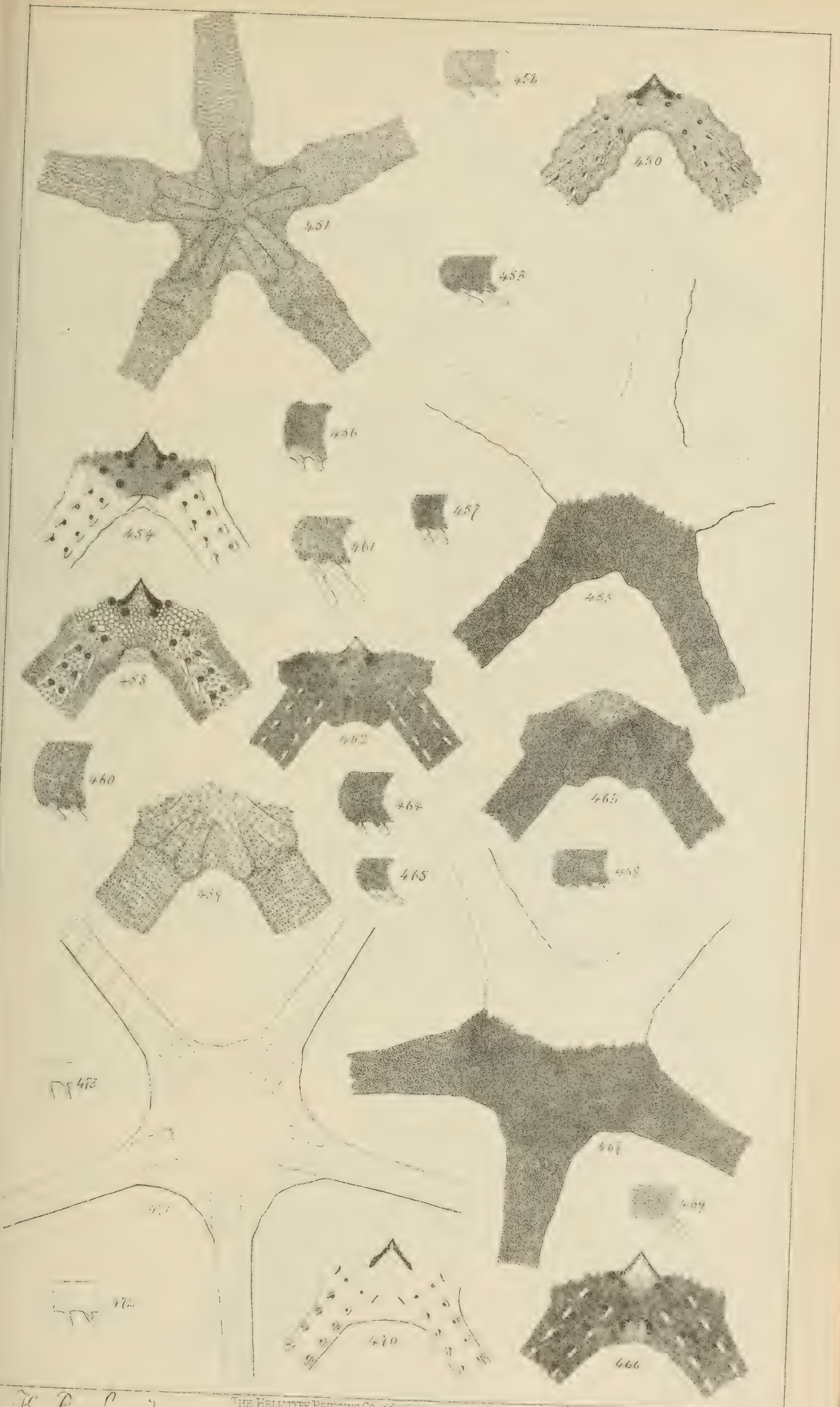
Plate XV



K. P. ad nat.

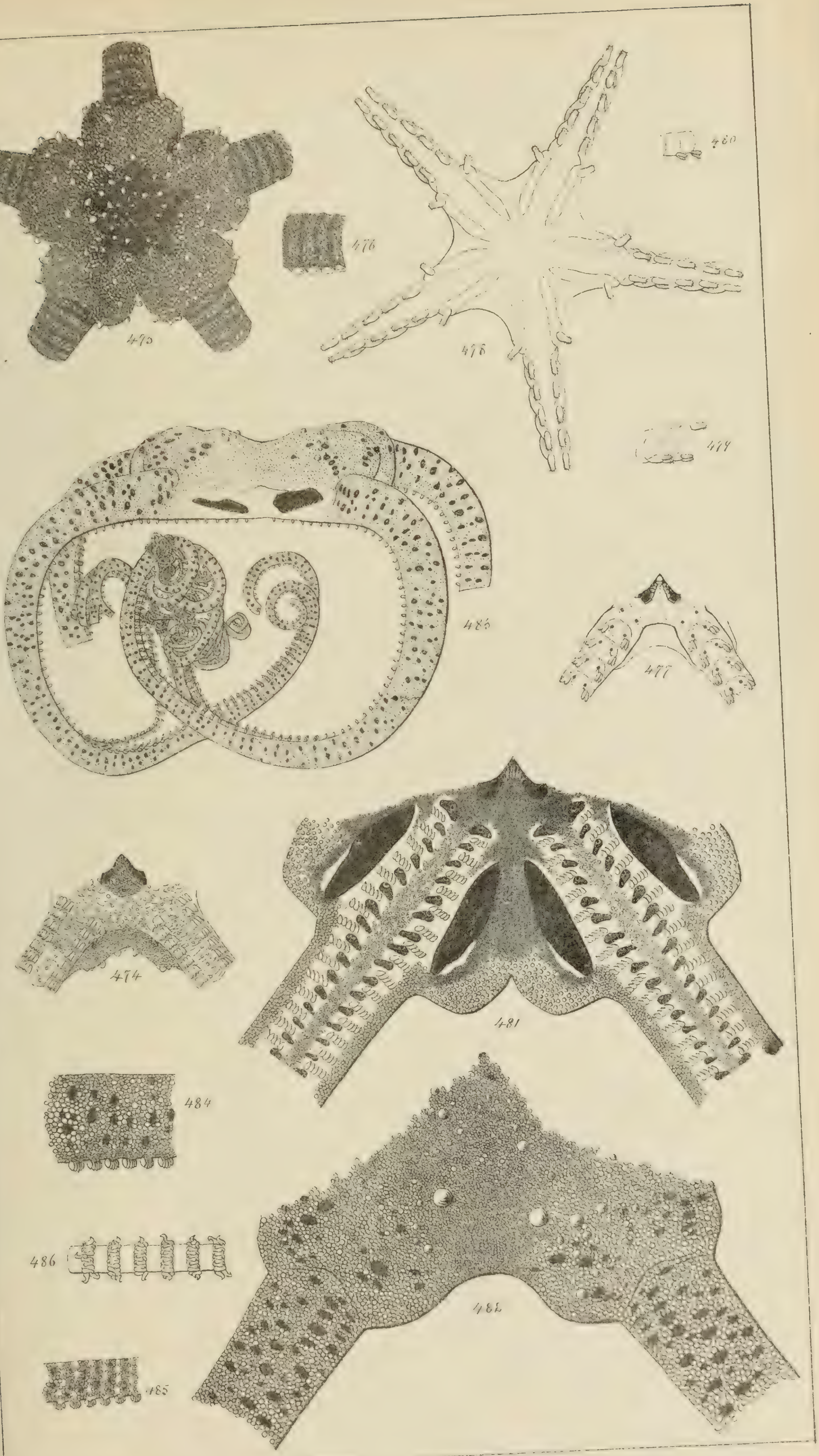
THE HELIOTYPE PRINTING CO. 220 DEVONSHIRE ST. BOSTON

Plate. XVI



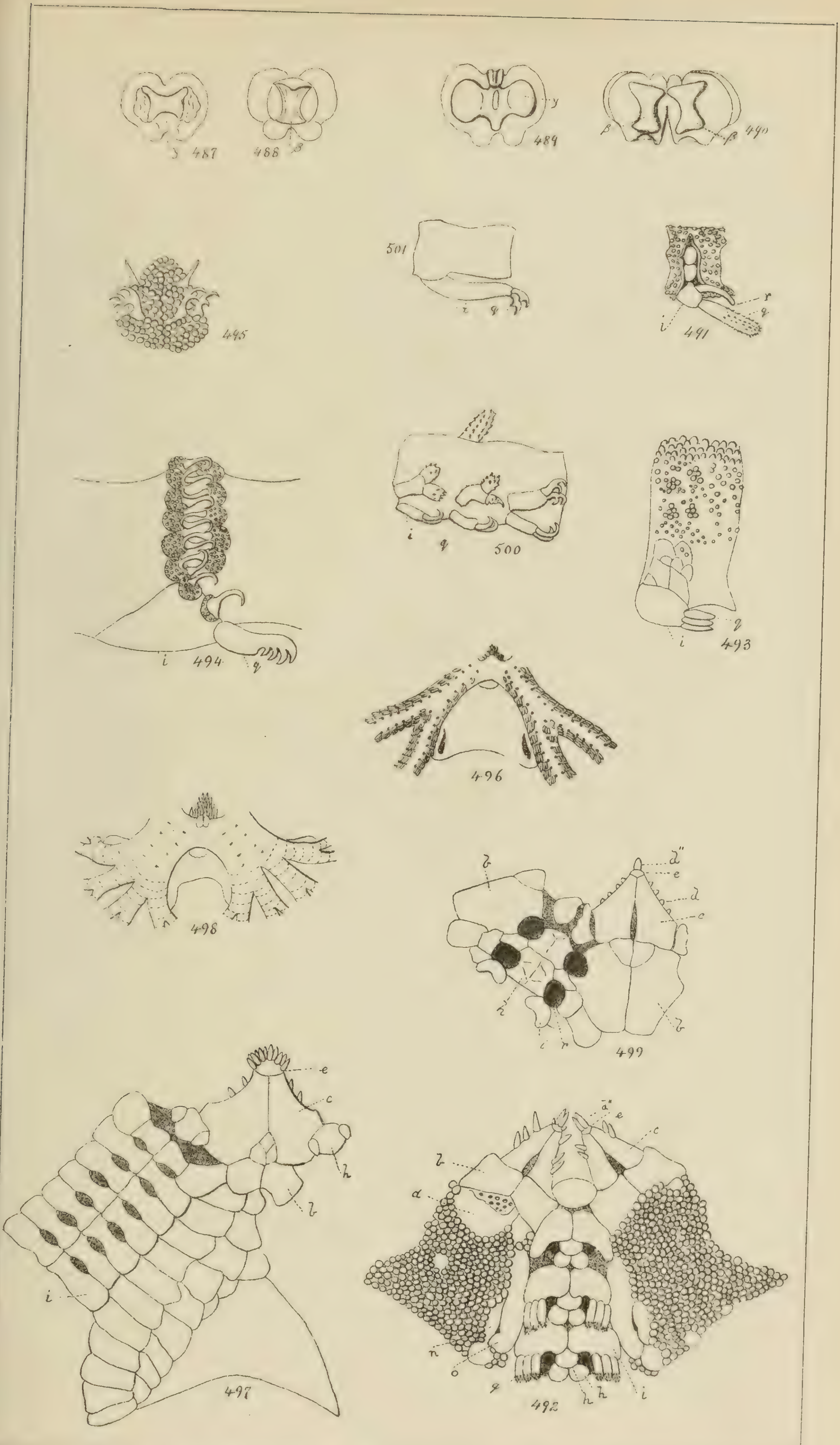
K. P. ad nat
THE HELIOTYPE PRINTING CO [illegible] SHIRE ST [illegible]

Plate XVII



THE HELIOTYPE PRINTING CO. 220 DEVONSHIRE ST. BOSTON.

Plate XVIII



J. L. ad nat.

THE HELIOTYPE PRINTING CO. 220 DEVONSHIRE ST. BOSTON

Plate XIX

No. 3. — *Reports on the Results of Dredging, under the Supervision of* ALEXANDER AGASSIZ, *in the Gulf of Mexico*, 1877–78, *by the United States Coast Survey Steamer "Blake,"* LIEUTENANT-COMMANDER C. D. SIGSBEE, U. S. N., *Commanding.*

(Published by permission of CARLILE P. PATTERSON, Supt. U. S. Coast Survey.)

V.

General Conclusions from a Preliminary Examination of the Mollusca, by W. H. DALL.

THE collection made by Mr. Agassiz and Lieut.-Com. Sigsbee on board the "Blake" contained about five hundred species, from which the Pteropods and some other groups have been excluded, as will be seen by the tables, leaving 462 species to be considered in this paper.

Of course the specific determination of all these forms is a task which must necessarily occupy a large amount of time and labor, and if that had been a necessary preliminary to a report of any kind I should have nothing to say on the subject at the present time. Fortunately, however, the generic affiliations can be approximately determined almost at sight, and species may be almost as readily separated from one another by a practiced eye; so that it is not necessary to wait for the completion of the drudgery of researches into the nomenclature of the various specific forms before announcing any general conclusions.

Before proceeding to these it is necessary to make a few preliminary statements.

I. The observations herein tabulated are not to be taken as exact in every instance. The limits of a species, or the reference to a subordinate generic group, is liable to be modified, occasionally, by more mature study. The examination of the collections for 1878–79, made under the supervision of Mr. Agassiz on board the "Blake," will doubtless add to, and in some instances change, the figures deduced from the collections of the previous season; all that is claimed for the conclusions here put forward is, that the general character of them seems already to be sufficiently established by the evidence in hand.

II. The combination of sundry shoaler-water collections, made by Pourtalès and Agassiz on the Coast Survey steamers "Bibb" and "Hassler," with the deep-sea dredgings, has proved of the highest importance, by completing the evidence in several cases where the absence of material from shoal water would have rendered a suspension of judgment necessary.

III. In several cases where the presence of dead shells in the deep-water material was the only evidence of the presence of a shoal-water species there, its living presence has not been taken as proved unless the multiplication of instances and graduation of depths confirmed the supposition. If a too great conservatism has been exercised in this way, it is on the side of safety in the generalizations. The names provisionally adopted in the tables are of a conservative character as regards their limits; since, in this way, a more just comparison with the lists of authors like D'Orbigny and C. B. Adams is rendered possible; and this course is also less likely to result in errors of determination due to insufficient study.

IV. The absence of any tolerably complete catalogue of West Indian mollusks in accessible shape has interfered with carrying the comparisons as far as might have been desired. The best that could be done was to compare the lists of C. B. Adams's Jamaican shells and those described in D'Orbigny and Sagra's Mollusca of Cuba, to eliminate identical species, and to assume that the resulting list bore about such a proportion to the whole litoral molluscan fauna of the West Indies as the "Blake" dredgings do toward the whole abyssal fauna. Upon this assumption, however, though so convenient for a brief comparison, no very important conclusions are based. As the shells quoted by the above-mentioned authors were all (or nearly all) obtained in the limits of the shore fauna, they afforded a better means of comparing that faunal region with the abyssal region than more modern and complete lists like that of the shells of Guadaloupe (Crosse and Fischer), which contains many true deep-water species brought up on fishing-lines or by coral-hunters.

The following are the most interesting and important deductions which seem to result from the facts before me.

I. The facts, already known, that certain species of mollusks have a very limited vertical range, forming respectively a litoral and an abyssal fauna, are supplemented by the additional hitherto unrecognized fact that a fair proportion (say 20 per cent in the present case) have a vertical range which extends from the true litoral region (less than 50

fathoms) to the depths of the abyssal region (250 to 2,000 fathoms) unlimited by temperatures actually encountered.

II. Of the species with great vertical range (from less than 100 to more than 500 fathoms), the smallest part (ten per cent) are of groups which have been regarded as belonging to or characteristic of the shores of cold or boreal areas. The next larger part (twenty per cent) belong to groups hitherto considered characteristic of shoaler warm or tropical waters, while more than sixty per cent belong to groups not especially characteristic of the *litorale* of either region.

III. Of the species found in the abyssal fauna without regard to their vertical range above it, ten per cent may be termed boreal, thirteen per cent tropical, and more than seventy-five per cent uncharacteristic forms.

IV. Since the tropical forms belong to the same groups as those characteristic of the local litoral mollusk fauna, it is eminently probable that the abyssal regions have local faunæ proper to their various portions, and that a universal exclusive abyssal fauna, so far as mollusks are concerned, does not exist. This must be qualified by the admission of the existence in the abysses (as well as on the *litorale*) of ubiquitous species-forms; which, however, do not form a universal abyssal fauna, any more than *Mytilus edulis*, *Saxicava rugosa*, and *Poronia rubra* form a universal litoral fauna. The local nature of different portions of the abyssal fauna is also confirmed by the distinctness of the Challenger mollusks from those of the Blake, but a very small number appearing identical as far as a cursory examination could determine.

There can be no doubt that the uniformity of generally low temperatures (and consequently of food) affords special facilities for the wide distribution of boreal forms through the abyssal region. But where adjacent shores can (by washing and sinking) afford a different or greater variety of food without too excessive temperatures, local abyssal faunæ will probably always be developed, and with characteristics assimilated to those of the litoral fauna of the same part of the earth's surface. The present collection shows conclusively that a difference in pressure of some 120 atmospheres and in temperature of 41.5 degrees has been sustained by different individuals of the same species without perceptible change in the external appearance of their hard parts or shells.

V. The specific characters of many of the strictly abyssal species appear to exhibit a very remarkable degree of variation within supposed specific limits, although it would seem as if the conditions under which they live must be remarkably uniform. This would indicate that the

tendency to variation is less dependent upon changes in the existing environment than has generally been assumed.

The total number of litoral species recorded by Adams and D'Orbigny, throwing out those groups, like the Pteropods, not germane to the inquiry, is 580, as compared with 461 collected by the Blake. The number of genera represented by the former is about 110, while some 98 genera are found in the Blake collection. The 461 species included in the last-mentioned collection comprise 210 which are litoral or do not reach great depths, while 251 are abyssal or ubiquitous. These numbers are of course approximate, and subject to correction, but probably not seriously in error.

Out of 48 species, of 44 genera, having great vertical range, 24 have a range of 500 to 750 fathoms; 17 have a range of from 750 to 1,000 fathoms; and 7 have a range of from 1,000 to 1,555 fathoms. Bearing in mind that the absolute depth of the extreme range may be much greater than this, the astonishing fact is evident that the same species may experience a difference, between two of its stations, of the weight of nearly two miles of sea-water. The possibility of this of course lies in the permeation of the soft parts by the sea-water, thus equalizing the pressure. It is almost certain, however, that individuals from the great depths would die if removed to shoaler water, unless by extremely slow degrees.

It is noticeable among the deep-sea forms that the sculpture tends to be slight, the shell thin, pale or colorless, and in the spiral shells there is a tendency to a knobbing or denticulation of the posterior edge of the whorls at the suture. To each of these peculiarities there are, however, conspicuous exceptions.

The following tables exhibit in detail the statistics from which the foregoing conclusions have been drawn.

COMPARATIVE TABLE OF THE LITORAL AND ABYSSAL WEST INDIAN AND GULF FAUNÆ.

	Group or genus.	D'Orb. and C. B. Ad.	Blake.	Vertical range of genus in fathoms.		Species belonging to the		Range of single species.	
		No. sp	No. sp.	From	To	Litor.	Abyss.	From	To
1	Anatinidæ	1	7	15	640	5	2	?	640
2	Ancillaria	1	0						
3	Anomia	2	0						
4	Arca	15	6	13	1568	3	3	310	1568
5	Astyris	(Columb.)	3	220	805	0	3	220	450
6	Avicula	3	0						
7	Bulla	17	4	37	1568	1	3	100	1568
8	Cadulus	0	6	30	1002	0	6	100	1002
9	Calyptræa	1	1	95	100	1	0	95	100
10	Cancellaria	2	1	54	84	1	0	54	84
11	Cardita	2	6	13	640	3	3	?	640
12	Cardium	10	9	30	187	7	2	30	182
13	Cassis	4	0						
14	Cerithiopsis	20	11	50	1002	2	9	100	1002
15	Cerithium		0						
16	Chama	2	1	80	100	1	0	80	100
17	Chitonidæ	12	1	128	?	0	1		
18	Cistella	0	2	80	805	0	2	30	805
19	Columbella	16	(Astyris.)						
20	Conus	4	3	19	100	3	0	19	100
21	Corbula	5	10	48	805	5	5	48 100	805 640
22	Crania	0	1	105	116	1?		105	116
23	Crassatella	0	1	30	?	1	0		
24	Crepidula	2	1	?	539	0	1		
25	Crucibulum	0	1	54	128	1	0	54	128
26	Cylichna		3	100	640	1	2		
27	Cypræa	6	0						
28	Cypricardia	1							
29	Dentalium	3	9	50	1568	2	7	50	1002
30	Dolium	2							
31	Donax	4							
32	Erato	1							
33	Erycina	1							
34	Eudesia		1	100	310	0	1	100	310
35	Eulima	4	10	100	640	6	4		
36	Fasciolaria	2	3	54	292	2	1	220	292
	Fissurellidæ								
37	*a.* Puncturella	14	2	100	640	0	2	100	640
38	*b.* Glyphis		3	50	805	1	2	100	805
39	*c.* Fissurella		1	13	805	0	1	13	805
40	*d.* Fissurellidea		1	100	?	1	0		
41	*e.* Emarginula	2	3	80	640	0	3	100	640
42	Fusus	3	5	15	1002	3	2	15	1002
43	Galerus	1	1	?	640	0	1		
44	Gastrochæna	2							
45	Gouldia	2	3	13	1568	0	3	13	1568
46	Hipponyx	3	1	229	?	0	1		
47	Leda	2	4	54	1568	0	4	54	1002

	Group or genus.	D'Orb. and C B. Ad.	Blake.	Vertical range of genus in fathoms.		Species belonging to the		Range of single species.	
		No. sp.	No. sp.	From	To	Litor.	Abyss.	From	To
48	Leptothyra		2	15	1002	1	1	15	1002
49	Lima	4	6	19	640	3	3	19	287
50	Limopsis		2	13	1568	0	2	30	1568
51	Liotia	1	2	80	220	1	1		
52	Lithodomus	4							
53	Litorina	17							
54	Lucinidæ	15	12	19	805	8	4	19	640
55	Lutraria	3							
56	Lyonsia	1	2	95	1920	1	1	?	1920
57	Mactra	2							
58	Margarita		6	80	1568	0	6	80	1568
59	Marginella	17	13	54	1002	8	5	54	1002
60	Mitra	6	4	84	119	4	0		
61	Modiola	9	1	220	339	0	1		
62	Modiolaria		2	339	640	0	2		
63	Monodonta	5	1	37	220	1	0		
64	Murex	7	5	54	640	1	4	54	640
65	Narica	3							
66	Nassa	13	3	13	640	2	1	13	640
67	Natica	7	4	14	640	2	2		
68	Neæra		10	54	1002	5	5	84	229
69	Nerita	6							
70	Neritina	7							
71	Nucula		3	30	640	0	3	30	640
72	Nuculocardia	1							
73	Odostomia	2							
74	Oliva	6	1	54	?	1	0		
75	Olivancillaria	1							
76	Olivella	6	4	72	805	0	4	127	805
77	Oniscia	2							
78	Orbicula	1							
79	Ostrea	4							
80	Ovula	2	2	72	80	2	0		
81	Patella	7							
82	Pecten	4	9	13	805	8	1	13	805
83	Pectunculus	5	2	54	888	1	1	54	888
84	Pedicularia		1	100	640	0	1	100	640
85	Perna	5							
86	Petricola	2							
87	Phasianella	4	1	287 ?		0	1 ?		
88	Pholas	6							
89	Pinna	4							
90	Planaxis	3							
91	Platidia		1	120	292	0	1	120	292
92	Pleurotomaria		2	69	200	1	1 ?		
	Pleurotomidæ								
93	*a.* Bela	} 25	{ 3	339	805	0	3	413	805
94	*b.* Drillia, etc.		108	15	1568	57	51	100	805
95	Plicatula	1	2	36	54	2	0		
96	Psammobia	4							
97	Purpura	12							
98	Pyramidella	1	1	84	100	1	0		
99	Pyrula	2							
100	Ranella	4							

	Group or genus.	D'Orb. and C. B. Ad.	Blake.	Vertical range of genus in fathoms.		Species belonging to the		Range of single species.	
		No. sp.	No. sp.	From	To	Litor.	Abyss.	From	To
101	Ringicula	1	1	339	640	0	1	339	640
102	Rissoa	10	6	100	640	3	3		
103	Rissoina	6							
104	Rotella	5							
105	Sanguinolaria	2							
106	Scaphander	0	1	54	220	0	1		
107	Scalaria	9	7	15	805	4	3	72	805 ?
108	Semele	4	2	30	127	2	0		
109	Seguenzia	0	1	292	?	0	1		
110	Sigaretus	3	1	84	?	1	0		
111	Siliquaria	0	2	80	805	1	1	80	805
112	Solarium	5	3	80	805	0	3	80	805
113	Solecurtus	3							
114	Solen	1							
115	Solenella	0	1	118	?	1	0		
116	Sphenia	3							
117	Spondylus	2							
118	Stomatia	1							
119	Strombus	6							
120	Tellinidæ	27	13	13	860	5	8	30	805
121	Terebra	2	4	14	640	3	1		
122	Terebratula	0	1	100	300	0	1	100	300
123	Terebratulina	0	1	30	805	0	1	30	805
124	Teredo	1							
125	Thecidium	0	2	100		0	2		
126	Tornatella	1	4	111	805	0	4	111	805
127	Trichotropis	0	1	84	?	1	0		
128	Triforis	0	2	80	175	1	1		
129	Tritonium	10	1	100	?	1	0		
130	Trivia	4	6	80	805	1	5	80	805
	Trochidæ								
131	*a.* Gibbula	9	3	54	805	0	3	54	805
132	*b.* Calliostoma		9	37	805	0	9	70	805
133	Turbinella	7							
134	Turbo	9							
135	Turbonilla	10	5	50	640	3	2		
136	Turritella	2	4	14	640	2	2		
137	Typhis	0	3	127	1002	0	3	127	1002
138	Utriculus	0	1	100	450	0	1	100	450
139	Veneridæ	18	17	30	805	14	3	84	287
140	Vermetus	3	6	37	805	3	3	37	805
141	Verticordia	1	5	84	310	2	3	100	310
142	Vitrinella	0	3	640	805	0	3		
143	Volvula	0	1	100	?	1	0		
144	Voluta	1							
	Waldheimia (see Eudesia)								
145	Xenophora	1	2	36	229	1 ?	1	36	229
146	Yoldia	0	3	182	1568	0	3	182	1568
	Totals	580	462			211	251		

Illustrations of the Range of individual species in Depth.

(*x*, boreal forms ; *o*, tropical forms ; *n*, uncharacteristic forms.)

o Arca, 100, 220, 310, 450, 480 fms.
o " (another sp.) 310, 1568 fms.
n Bulla, 100, 450, 533, 568, 640, 805, 1568 fms.
n Cadulus, 100, 31, 805, 1002 fms.
n Cerithiopsis, 100, 640, 805, 1002 fms.
n " (another sp.) 50, 85, 94, 100, 450, 805 fms.
n Cistella, 30, 43, 80, 100, 101, 200, 220, 250, 450, 640, 805 fms.
n Corbula, 100, 640 fms.
n " (another sp.) 48, 72, 100, 127, 450, 805 fms.
n Dentalium, 50, 80, 84, 100, 101, 119, 175, 200, 539, 640, 888, 1002 fms.
n " (another sp.) 339, 539, 640, 805, 860, 1568 fms.
n Eudesia, 110, 119, 125, 175, 200, 229, 310 fms.
Fissurellidæ.
x Puncturella, 100, 220, 640 fms.
n Glyphis, 100, 287, 805 fms.
n Fissurella, 13, 50, 80, 72, 127, 640, 805 fms.
n Emarginula, 100, 287, 292, 310, 640 fms.
n Fusus, 15, 54, 125, 128, 152, 229, 1002 fms.
n Gouldia, 13, 84, 310, 1568 fms.
n Leda, 54, 75, 80, 100, 190, 220, 287, 310, 640, 1002 fms.
x Limopsis, 30, 84, 100, 119, 220, 292, 310, 447, 450, 480, 539, 640, 805, 1568 fms.
n Lucina, 19, 84, 640 fms.
x Margarita, 100, 177, 220, 287, 331, 450, 539, 640, 805, 860, 888 fms.
x " (another sp.) 80, 119, 310, 1568 fms.
o Marginella, 54, 70, 72, 80, 100, 111, 125, 152, 229, 640, 805, 1002 fms.
o Murex, 54, 640 fms.
n Nassa, 13, 72, 80, 100, 177, 640 fms.
n Neæra, 84, 152, 229 fms.
n Nucula, 30, 84, 100, 158, 182, 220, 310, 539, 640 fms.
o Olivella, 127, 177, 805 fms.
n Pecten, 13, 30, 100, 119, 127, 229, 243, 287, 292, 310, 331, 424, 450, 480, 539, 640, 804, 805 fms.
n Pectunculus, 54, 68, 80, 84, 95, 119, 888 fms.
n Pedicularia, 100, 450, 640 fms., on Gorgoniæ and corals.
Pleurotomidæ.
x Bela, 413, 447, 640, 805 fms.
o Candelabrum, 100, 640, 805 fms.
o Ringicula, 339, 447, 640 fms.

o **Siliquaria**, 80, 100, 127, 220, 450, 805 fms.
o **Solariidæ**, (sp.) 101, 119, 128, 292 fms.
o " (another sp.) 80 – 805 fms.
n **Tellina**, 30, 54, 72, 80, 84, 111, 229, 805 fms.
n **Terebratula**, 100, 101, 119, 175, 270, 292 fms.
n **Terebratulina**, 30, 80, 100, 101, 115, 119, 127, 220, 240, 270, 292, 450, 471, 539, 640, 805 fms.
n **Tornatella**, 111, 310, 450, 805 fms.
o **Trivia**, 100, 119, 287, 640 fms.
o " (another sp.) 80, 175, 805 fms.
o " (another sp.) 80, 177, 640, 805 fms.
Trochidæ.
n **Gibbula**, 54, 80, 100, 220, 805 fms.
n **Calliostoma**, 70, 80, 100, 128, 805 fms.
o **Typhis**, 127, 158, 182, 175, 1002 fms.
n **Utriculus**, 100, 220, 450 fms.
n **Vermetus**, 37, 80, 95, 100, 805 fms.
x **Yoldia**, 182, 190, 450, 805, 1568 fms.

Ten distinctively tropical genera with fourteen species.

Five distinctively arctic genera with six species.

Twenty-eight uncharacteristic (or generally temperate-region) genera with thirty-one species.

Total species ranging from litoral to abyssal fauna, and which may be confidently quoted, fifty-one, of forty-three genera.

FEBRUARY 5, 1880.

No. 4.—*Reports on the Results of Dredging, under the Supervision of* ALEXANDER AGASSIZ, *in the Caribbean Sea,* 1878–79, *by the United States Coast Survey Steamer "Blake,"* COMMANDER J. R. BARTLETT, U. S. N., *Commanding.*

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VI.

Report on the Corals and Antipatharia, by L. F. POURTALÈS.

CORALS.

ALTHOUGH very rich in the number of specimens, the collections made by Mr. Agassiz in the dredging season of 1878–79 have added but few new species. Considering the extent of the ground covered (see Bull. Mus. Comp. Zoöl., Vol. VI. No. 1), we may assume that but little will be added hereafter to our knowledge of the association of corals forming the West Indian deep-sea fauna. No other region of the ocean-bottom has yielded so abundant a harvest, and we have therefore no sufficiently complete data for comparisons with regard to geographical distribution. But for the bathymetrical distribution, and its bearing on the determination of the probable depth in which strata of former ages, containing corals, were deposited, the material brought together in the series of papers of which this forms the last * will no doubt prove of some importance.

The following table is a recapitulation of all the species described,

* Deep-Sea Corals, by L. F. de Pourtalès, Illust. Cat. Mus. Comp. Zoöl., No. IV. Zoölog. Results of the Hassler Exped., by A. Agassiz and L. F. de Pourtalès, Ibid., No. VIII. Part 1. Report on the Dredging Oper. of the U. S. C. S. St. "Blake," by A. Agassiz, L. F. de Pourtalès, and T. Lyman, Bull. Mus. Comp. Zoöl., Vol. V. No. 9.

Compare also, for deep-sea corals of the West Indies, G. Lindström, Contributions to the Actinology of the Atlantic Ocean, Kongl. Svensk. Vetensk. Handl., B. XIV. No. 6; and H. N. Moseley, Preliminary Report on the True Corals dredged by H. M. S. "Challenger," &c., Proc. R. S., No. 170, 1875. Of Mr. Moseley's final report I have seen, at the time of writing this, only advance copies of the plates, for which, and for valuable communications, I am indebted to the author.

with their range in depth, and comparisons with other seas, and European and West Indian fossil faunæ: —

Species.*	Range in Depth.	Species common to West Indian and European Seas.	Genera common to West Indian Tertiary and W. I. Deep-sea.	Species common to W. I. Deep-sea and Sicilian Miocene.
	Fathoms.			
Caryophyllia berteriana	56–442		1	
" antillarum	82–994			
" cornuformis	209–450	1		×
" communis	127–892	1		1
" maculata	30–88			
" polygona	860			
Stenocyathus vermiformis	191–460			
Thecocyathus cylindraceus	84–315			
" lævigatus	100–315			
" recurvatus	175			
Trochocyathus Rawsonii	82–805			
" coronatus	333–861			
Deltocyathus italicus	60–888	1		1
Stephanocyathus elegans	209–288			1
" variabilis	476			1
Leptocyathus Stimpsonii	60–450	1		
Paracyathus DeFilippii	36–805		1	
" laxus	92–164			
" flos	100			
Turbinolia corbicula	100–220			
Stephanotrochus diadema	734–1200			
Schizocyathus fissilis	56–450	1		
Ceratotrochus typus	250–400		1	1
Flabellum Moseleyi	118–476		1	
" angulare	888			
Desmophyllum crista-galli	309–805	1		×
" Cailleti	73–1131			
" Riisei	88–120			
" solidum	315			×
Rhizotrochus fragilis	84–119			
" tulipa	84–175			
Lophohelia prolifera	195–874	1		1
" exigua	36–287			
Amphihelia oculata	158–892	1		1
Madracis asperula	36–180			
Axohelia mirabilis	56–287			
Lophosmilia rotundifolia	42–163			
Dasmosmilia variegata	60–164			
" Lymani	70–147			
Montlivaultia poculum				
Antillia explanata	75		1	
Parasmilia fecunda	68–450		1	
Asterosmilia prolifera	45–94		1	
Solenosmilia variabilis	120–805	1		
Cylicia inflata	100–242			

* Duncan has 35 species of simple and probably deep-sea corals from all the West Indian formations.

× signifies different but closely allied species.

Species.	Range in Depth.	Species common to West Indian and European Seas.	Genera common to West Indian Tertiary and W. I. Deep-sea.	Species common to W. I. Deep-sea and Sicilian Miocene.
	Fathoms.			
Colangia simplex	80–100			
Balanophyllia floridana	26–100	. . .	. . .	×
" palifera	36–458			
Thecopsammia socialis	195–262	. 1		
" tintinnabulum	120–539			
Trochopsammia infundibulum	291–805			
Dendrophyllia Goësi	250–400			
" alternata	150–189			
" cyathoides	270			
" cornucopia	73–400	. . .	. . .	×
Stereopsammia rostrata	164–805			
" profunda	539–805			
Bathyactis symmetrica	116–805	. 1		
Diaseris crispa	119–189	. 1		
" pusilla	119–189			
Guynia annulata	100–357	. 1		
Duncania barbadensis	103–191			
Haplophyllia paradoxa	324			
Anthemiphyllia patera	250–400			
Total, — 64 species		13	7	12

The total of sixty-four species is nearly as large as the total of the shoal-water or reef corals of the same region, if we reduce the number of the latter, as given by Duchassaing and Michelotti, to its proper proportions by the rejection of merely nominal species.

The proportion of simple forms to compound ones is very large, — fifty of the former to fourteen of the latter. The compound ones belong mostly to the families of Oculinidæ, Stylophoridæ, and Eupsammidæ, with one species each from the Eusmilinæ and Astrangiaceæ.

Comparing this association with the one prevailing in the same seas in shoal or moderately deep water, we find that there is not a single species in common to both, and that they are separated by an almost barren narrow zone. We find also that there is not a single simple species in the shoal-water fauna, and that the compound forms belong to the families of Astræidæ, Oculinidæ, Fungidæ, and Madreporidæ, the former preponderating by far. There are no Eupsammidæ. The nearest approaches between the two horizons would be as follows: *Madrepora cervicornis* dredged living by myself in Barbados in 17 fathoms, *Orbicella cavernosa* in 15 fathoms in Florida, and *Mycedium fragile* in 43 fathoms. The latter species comes in contact with the

following deep-sea forms: *Paracyathus DeFilippii, Caryophyllia maculata, Lophohelia exigua, Madracis asperula, Lophosmilia rotundifolia, Asterosmilia prolifera, Balanophyllia floridana* and *palifera*, of which the upper limit is between 30 and 40 fathoms.

For other seas the case would be somewhat different. In the Pacific and Indian Oceans, for instance, simple corals, as Flabellum and some other Turbinolidæ, some Balanophylliæ, and numerous large Fungiæ, occupy the shoal or moderately shoal water region, and the prevailing families are different also.

Nevertheless, the West Indian bathymetrical distribution seems to offer a fair criterion for the approximate determination of the depth at which some of the strata not lower than the cretaceous have been deposited. In older formations the forms are too different for comparison.

We can thus safely say that some of the miocene, pliocene, and pleistocene strata of Messina, of which the fossils have been so carefully described by Seguenza, were deposited in a depth averaging 450 fathoms, and ranging from abont 200 to 700 fathoms. This average is deduced from the eight principal species. Two species of Eupsammidæ would however give considerably less. The species identical, or very nearly related, used for this result, are given in the table; some of them still inhabit the Mediterranean, but others have been found living only in the West Indian deep-sea. Some of the miocene beds of the vicinity of Turin are also deep-sea deposits.

In the neighborhood of Vienna it is easy to see by means of Reuss's excellent monographs that great fluctuations of depth have taken place between the deposition of the different strata. The tables appended to Reuss's Memoir on the Austro-Hungarian Miocene show very well that the beds called "Oberer Tegel," for instance, in which there are Astræans in abundance allied with Porites, are shoal-water deposits; and that the strata called "Badener Tegel," particularly at Ruditz, were formed on the bottom of deep water, the corals found in them being chiefly Turbinolidæ, Oculinidæ, and Eupsammidæ.

With regard to the West Indian tertiaries, and more particularly the miocene beds, a careful discrimination of the corals of the different strata, such as we have in the papers of Reuss and Seguenza, seems to be still wanting. Reef corals and solitary species are quoted as from the same localities in Prof. Duncan's papers, and in a fine collection from San Domingo, presented to the Museum by the late W. M. Gabb. In the latter the different matrix in which some of the specimens are

imbedded points directly to different beds. Prof. Duncan's statement, that on some islands, such as Antigua and Trinidad, only reef species are found, shows also pretty conclusively that in other places there must be deep-sea deposits which were not brought to light here.

It is rather puzzling to find the West Indian miocene solitary species so different from the living ones. Even when belonging to the same genera (see table) the species of the miocene are of a very massive type, very different from the living; such are the Antilliæ and Asterosmiliæ, with one exception. It is possible that these massive forms, of which we have no analogous examples at the present time, may have been living in the shoal-water, protected by reefs in the same way as the Fungiæ, or some of the unattached compound corals, as Manicina or Isophyllia.

The effect of slow changes of level has to be considered also as a possible cause of mixture of the dead of one level with the living of another.

Caryophyllia berteriana Duch.

Caryophyllia formosa Pourt.

The more the number of specimens of these forms accumulate, the greater the difficulty to separate them. The principal difference consists in the form of the septa, very exsert in *C. berteriana*, very little so in *C. formosa*, but I now find many intermediate forms. The number of the pali, 12 to 16, cannot well be retained as of specific value.

Duchassaing has described as new *C. sinuosa*, *corona*, and *protei*, but the descriptions either apply to young specimens or to mere varieties of the older described species.

There is in the collection a specimen taken in the act of swallowing a small fish, which is partly inside the mouth, with the buccal membrane stretched from both sides over its middle.

Range * from 56 to 442 fathoms, in 19 stations, off Santa Cruz, Montserrat, Guadeloupe, Dominica, Martinique, St. Vincent, the Grenadines, Grenada, and Barbados.

Caryophyllia cornuformis Pourt.

Old specimens show considerable anomaly in the arrangement of the pali, which are wanting sometimes in nearly one half of the calicle, and their place filled up by enlarged ribbons of the columella.

A case of involuntary parasitism, like the one mentioned in "Deep-Sea

* The range in depth in this and the following descriptions refers only to this year's work.

Corals," was found in this collection. The coral fastened among the pebbles with which a Phorus has ornamented his shell appears to have flourished remarkably well in that position, as indeed it ought to, having been carried about in search of food, and prevented from sinking in the mud.

Range from 200 to 400 fathoms, off Havana and Barbados.

Caryophyllia antillarum Pourt.

Range from 82 to 994 fathoms, in 11 stations, off Nuevitas Cuba, Montserrat, Guadeloupe, Grenadines, Grenada, and Barbados.

Caryophyllia communis Mos., *var.* costata.

Ceratocyathus zancleus Seg.

Plate I. Figs. 12 and 13.

I agree with Dr. Duncan and Mr. Moseley in joining the genus Ceratocyathus* of Seguenza to Caryophyllia. The numerous species described by that author pass gradually into each other. At one end of the series we have the forms with nearly equal septa and costæ, like *C. communis;* at the other those which have them very unequal, like *C. zancleus.* All our specimens are extreme forms, still more marked in the inequality of septa and costæ than the last mentioned.

As Seguenza has a *Caryophyllia zanclea* as well as a *Ceratocyathus zancleus,* that name could not well be retained without confusion ; *Ceratocyathus costatus,* however, is the same.

A series of fine specimens shows the mode of growth. The young are erect, with a thin peduncle attached to a small pebble or shell ; as it grows in height, the support not being sufficient, it falls over on its broadest side, and the tendency to grow upward, and to keep the calicle above the mud, produces the curved base. The curvature of those corals which have it in the plane of the smaller diameter is thus easily explained. It is more difficult to understand the curvature in the plane of the greater axis, which is the rule in some species of corals.

The largest specimen measures 36 mm. on the greater diameter of the calicle, and 31 on the smaller. None of our specimens have more than 16 pali; the number can rise to 24, according to Seguenza and Moseley.

Some of the young specimens are still straight, and resemble very much Moseley's *Acanthocyathus spinicarens.*

Range from 127 to 892 fathoms, in 13 stations, off Santa Cruz, Saba Bank, St. Kitts, Guadeloupe, Dominica, Martinique, St. Vincent, the Grenadines, Grenada, and Barbados.

* See under the head of Asterosmilia, in "Deep-Sea Corals," a rectification of my remarks on this genus.

Caryophyllia maculata Mos.

Bathycyathus maculatus Pourt.

Mr. Moseley has, I think, rightly placed Bathycyathus in the genus Caryophyllia.

In 88 fathoms, off Montserrat.

Stenocyathus vermiformis Pourt.

Plate I. Figs. 15, 16.

I cannot find in my specimens the dissepiments mentioned by Mr. Lindström. His figures represent sections near the centre and near the exterior wall, where there are connecting bands; but I have always found the central part of the chambers unobstructed from end to end. In Pl. I. Fig. 15, I have given a figure of a decalcified specimen, showing the complete cast of the inside cavity; the outside is entirely uninterrupted by any dissepiments, and shows only the regular little knobs formed by the contents of the small cavities in the wall. In Fig. 16 two of the segments are separated so as to show the bands connecting them between the pali and the septa.

Range from 191 to 400 fathoms, in two stations, off Havana and Martinique.

Thecocyathus cylindraceus Pourt.

Range from 84 to 250 fathoms, in three stations, off Havana and Barbados.

Trochocyathus Rawsonii Pourt.

Specimens fixed by a rather large base are the most common form, free individuals being comparatively rare. When fixed, there is not much difference from the genus Paracyathus, unless it is the more regular pali.

Range from 82 to 221 fathoms, in four stations, off Grenada and Barbados.

Trochocyathus coronatus Pourt.

Odontocyathus coronatus Mos.

Very fine specimens, both living and dead, were obtained, but no young ones.

Range from 333 to 861 fathoms, in four stations, off Virgin Gorda, Dominica, and Grenada.

Deltocyathus italicus Edw. & H.

Plate I. Figs. 1-8.

I have adopted the name of the original fossil species for the exceedingly variable living forms, as Prof. Duncan has done before, the differences between the living and the fossil being less than between the different living varieties. The living attain a larger size than the fossil forms.

About two hundred and fifty specimens of the different varieties of this species were obtained, so that from the various expeditions we must have about a thousand specimens. Among that number I have not found a single one attached, or showing marks of adherence.

There are four well-marked forms, more or less connected by intermediate ones, though, on the whole, specimens showing the passage from one form to another are rare, and one form generally prevails in each particular dredging.

Variety α, *Agassizii*. Pl. I. Fig. 2.

The typical form on which I based my first description, equicostate, with more or less conical base, costæ rather prominent, sharp, finely serrate and granulate. (Deep-Sea Corals, Pl. II. Figs. 1, 2, 3. Lindström, Contr. to Actinol. of Atl. Oc., Pl. II. Figs. 15, 16, 17. Moseley, Madrep. of the Challenger Exped., Pl. II. Figs. 2 and 3.) This form approaches nearest *D. italicus;* there are specimens in which the spines of the costæ are large and blunt, and resemble the bead-like grains of the fossil species. (Pl. I. Fig. 3.) This was considered by Reuss and by myself as the chief specific difference between the fossil and living forms. The variety *australiensis*, Duncan, does not differ particularly from this one; but *D. orientalis*, Duncan, is different. The corals referred to this genus by Mr. Tenison-Woods do not appear to me to belong here, as far as the very rough figures allow me to judge.

Variety β. Pl. I. Fig. 4.

Like the preceding in most particulars, but more hemispherical in outline; the costæ are a little more differentiated, and the primaries have sometimes a slight enlargement or blunt knob in the middle of their length, showing a tendency at one period of their growth to form the points characteristic of the next form, or variety *calcar*. The pali in this form are not very prominent, and the junction of the septa of younger with those of older cycles occurs far down towards the centre of the calicle. Some specimens resemble very much *Trochocyathus obesus*, one specimen of which from Tortone, Italy, in the Museum collection, has likewise only one knob or blunt spine on the primary costæ.

Variety γ, *calcar*. Pl. I. Fig. 5.

(Deep-Sea Corals, Pl. II. Figs. 4 and 5, and Pl. V. Figs. 9 and 10; and Hassler Exp., Pl. VI. Fig. 11. Lindström, l. c., Pl. I. Fig. 13, Pl. II. Figs. 14, 18, 19.) I give the figure (Pl. I. Fig. 1) of the specimen, which has induced me to consider this only a variety of the other form. Besides the specimen with double horns figured in Deep-Sea Corals (Pl. V. Fig. 9), there is another one in the present set with three or four horns on the primary costæ, apparently the result of an injury. Reuss mentions a specimen of *D. italicus* with remarkably thickened, and almost lamelliform, primary and secondary septa, so that it seems that the fossil form had also the tendency to vary in the same manner.

Variety δ.—Pl. I. Figs. 6, 7, 8.

Base nearly flat, with a small umbo in the centre, hardly marked in most specimens, outline of section somewhat re-entering above, so that the diameter of the calicle is somewhat less than the diameter of the base. Costæ equal, flat, thick, and contiguous, separated only by a linear though rather deep furrow, finely granulated, and having a faint granular keel. The pali are thick, and the columella oblong, composed of thick pillars, and well separated from the pali; the septa are thickened at the outer borders, covered laterally by short thick spines. All the specimens are uniformly white, without purplish spots. Moseley's *D. magnificus* resembles it in outline, but has not the same costæ, septa, and columella. I have seen no passages between this form and the others, when adult, but in the young the costæ are thin, and not essentially different from variety α.

Depth seems to have no influence on the prevalence of one or the other form.

In the polyps the outer sphincter when contracted covers the tentacles which are drawn back in the notch between the septa and pali. The office of the pali seems to be partly to support the buccal membrane, partly to protect the retracted tentacle. They never support extra tentacles in any of the corals in which I have had the chance to observe the well-preserved polyp.

Range from 73 to 878 fathoms in 39 stations, off Havana, in Old Bahama Channel, off Santa Cruz, Virgin Gorda, St. Kitts, Montserrat, Guadeloupe, Dominica, Martinique, St. Lucia, St. Vincent, the Grenadines, Grenada, and Barbados.

Stephanocyathus elegans Seguenza.

Three fine specimens of this species were obtained, two living and one dead. They agree most with Seguenza's variety *subspinosus*.

The second lobe of the pali, generic character according to Seguenza, resembles the parts of the columella, but is well separated from it; it is not constant, and distinct only in the second or third order. The columella, which is formed of twelve or more blunt prongs, is in younger specimens more diffuse. The septa of the fourth cycle unite through their pali with those of the third, and the latter with the second. The septa of the fifth cycle are small, and reach only half-way to the centre.

The polyp has stout tentacles; in one specimen those of the three first cycles were white, the others dark purple, with white tips; in the other they were all purple, those of the youngest order least so. They are disposed in several circles, and do not appear to be very retractile. The outer sphincter is not distinctly marked. The buccal membrane is very thin, and in both specimens many of the pali had pierced it.

Range from 209 to 288 fathoms, in three stations off Barbados.

Stephanocyathus variabilis Seguenza.

Plate II. Fig. 2.

Two specimens, one living and one dead, are referred to this species. They are both larger than those figured by Seguenza, and differ slightly from his description. There is no plicated rudimentary epitheca, and the costæ remain very flat and indistinct to the edge of the calicle, instead of becoming prominent and cristiform. Otherwise there are no essential differences.

The polyp seems to have been rather highly colored, dark purplish in alcohol in all its parts except the larger tentacles, which are whitish. Tentacles stout, forming a double circle at a considerable distance from the mouth. Buccal disk radially plicated, and very tough. Diameter of largest specimen, 48 mm. Height, exclusive of primary and secondary septa, 8 mm. Height of primary septa, 5 mm.

In 476 fathoms, off Martinique.

Leptocyathus Stimpsonii Pourt.

Both the long and the short varieties.

Range from 92 to 400 fathoms, in two stations, off Havana and Grenada.

Stephanotrochus diadema Moseley.

Plate II. Fig. 1.

One living and two dead specimens. The tentacles were remarkably full of nematocysts, and must have been quite long. Those of the three first orders are not very different from each other in size, and about equidistant from the centre; those of the fourth and fifth are smaller and farther removed. The tentacular circle is quite distant from the mouth, having a large bare plicated buccal disk.

In 734 fathoms off Guadeloupe, and 1,200 fathoms in lat. 19° 7′ N., and long. 74° 52′ W.

Schizocyathus fissilis Pourt.

Range from 56 to 170 fathoms, in seven stations, off Martinique, St. Lucia, Grenada, and Barbadoes.

Paracyathus laxus n. sp.

Plate I. Figs. 9–11.

Corallum turbinate, turgid, short-pedicellate, sometimes becoming free when attached to a small object, which then becomes covered up by an epithecal growth. Generally dark-colored. Costæ not prominent, finely granulated, and separated by a fine linear convex ridge. They are covered by a very thin rudimentary epitheca, through which the granulations can be seen.

The calicle is circular, with a moderately deep fossa. The septa are somewhat exsert, thin, rounded, with granules on the sides, arranged in rows parallel to the edge. Four cycles and part of the fifth in most of the systems, which are thus somewhat unequal. Pali prominent and large, rather irregular, sometimes two or three lobed ; columella much looser than in the other West India species. The young have a very loose structure, with thin lamellar pali, and rudimentary columella not yet developed into pillars.

Largest specimens, 30 mm. high ; diameter of calicle, 19 mm.

Range from 88 to 164 fathoms, in four stations, off Montserrat, Martinique, and Grenada.

Paracyathus DeFilippii Duch. & Mich.

Very variable species, which may in the end be found identical with the Mediterranean species, *P. striatus* and *pulchellus*. I have had no opportunity for direct comparison.

Range from 56 to 458 fathoms, in sixteen stations, off Santa Cruz, St. Kitts, Montserrat, Dominica, Grenada, Bequia, and Barbados.

Ceratotrochus typus Pourt.

Conotrochus typus Seg.
Ceratotrochus hispidus Pourt.

A few more specimens obtained make it evident that the two corals above named are the same. I have before me specimens entirely or partially covered by an epitheca, or completely destitute of it. The last I have called *Ceratotrochus hispidus*. The genus Conotrochus of Seguenza, also adopted by Reuss and Duncan, must necessarily be dropped, since it differs from Ceratotrochus only by the presence of an epitheca. *Ceratotrochus multispinosus* has, according to M.-Edwards and Haime, a partial epitheca ; and, looking over a series of specimens from the Italian tertiary, I find that the character is just as variable, the epitheca being total, partial, or absent.

It is rather unfortunate to have to retain Seguenza's specific name for a species not very typical in its characters.

Range from 250 to 400 fathoms, in two stations, off Havana and St. Kitts.

Flabellum Moseleyi Pourt.

Plate II. Figs. 13 and 14.

Corallum with a rather long and slender peduncle, strongly curved in the plane of the smaller diameter; a scar of attachment at the end. Calicle widely open, elliptical, diameters as 1 to 1.3. Margin horizontal. Costæ of the first and second order about equal, forming stout more or less knotty ridges ; the lateral ones not very different from the others, except in the younger stages, where they are cristiform. The other costæ are represented

by mere shallow furrows. Angle of aperture about 40°. The whole surface is marked with chevron-shaped lines of growth; in one specimen the whole surface is finely granulated, in the others it is smooth and shiny, as usual in the genus. The septa are in six systems and five complete cycles. They are marked with fine radiating granulated ridges. The primaries and secondaries are about equal, and very exsert; all the others remain below the border of the calicle, which is deeply indented. There is a callous thickening at the base of the septa, in the bottom of the fossa. The color of the corallum is a dirty flesh-color, inside and out. The tentacles are equal in number to the septa; I do not find the small supernumerary ones noticed by Moseley in *Fl. alabastrum*. There appears to be no outer sphincter to cover them.

The diameters of the largest specimen are 5 and 4 cm.

It is somewhat related to Moseley's *Fl. alabastrum*, but differs from it by its elliptical outline, long and curved peduncle, and horizontal margin.

Five fine living specimens were obtained.

Range from 118 to 476 fathoms, in six stations, off Dominica, Martinique, Grenada, and Barbados.

Desmophyllum Riisei Duch. & Mich.

Thalamophyllia Riisei Duch.

Plate I. Fig. 14.

This is a true Desmophyllum, differing from the typical ones in growing always in clusters from an incrusting base. It is hardly necessary to form a new genus for it on that account. I can find no dissepiments, as stated by Duchassaing.

The corallites are much longer and narrower than in the figure of Duchassaing and Michelotti. They are generally 2 or 3 cm. high, with a diameter of only 5 to 7 mm.

Range from 88 to 120 fathoms, in five stations, off Montserrat, Dominica, and Martinique.

Desmophyllum crista-galli Edw. & H.

In 399 and 442 fathoms, off Martinique and Barbados.

Desmophyllum Cailleti Duch. & Mich.

Range from 73 to 1,131 fathoms, in eighteen stations, off Havana, Nuevitas Montserrat, Guadeloupe, Dominica, St. Lucia, St. Vincent, and Barbados.

Rhizotrochus tulipa Pourt.

Range from 84 to 106 fathoms, in three stations, off Barbados.

Lophohelia prolifera Edw. & H.

A variety with strongly marked primary and secondary costæ.

In 291 fathoms off Grenada, and in 874 fathoms in lat. 17° 47′ N., and long. 67° 3′ W.

Amphihelia oculata Edw. & H.

Numerous specimens, showing much variation. The most common form agrees exactly with Prof. Duncan's Figs. 1, 2, and 3 of Pl. XLV. of the "Porcupine" Madreporaria, and is similarly deformed by a parasitic annelid; this has apparently the tendency to smooth out the striæ. Another variety, free of parasites, grows into long branches, with alternate calicles, forming regular zigzags. Often each calicle gives out two opposite ones, one of them forming the main branch, the other beginning a side branch on the same pattern. Prof. Duncan's Fig. 1, Pl. XLVI., shows this mode of growth, but not with the regularity of some of our specimens. It has also been represented by Seguenza and by Moseley. Old branches of this form become much thickened and compressed. Both forms are the same, since they are found in the same specimen. The striæ vary much, and I doubt if they present a sufficient character to separate *A. oculata* and *ramea. Amphihelia sculpta*, Seg., to which I referred specimens dredged last year, is the same.

Range from 164 to 892 fathoms, in seven stations, off Guadeloupe, Dominica, Martinique, Grenadines, and Grenada.

Axohelia mirabilis Duch. & Mich.

Very common, and rather variable. None were found agreeing with *Axohelia Schrammi*; but those I had identified as *A. myriaster* and *A. dumetosa* I have now good reason to believe are only differences of age. Old specimens are generally coarsely striated, somewhat like the figure of *A. myriaster* given by M.-Edwards and Haime; while younger branches are mostly granulated. As *Axohelia myriaster* is an East Indian species, I shall use provisionally the name first used by Duchassaing and Michelotti for the West Indian species; but having seen no specimen of the former, I cannot tell in what they differ.

Among the varieties there is one with slender branches and calicles, raised on conical projections, as in *Oculina varicosa.* Specimens obtained from the telegraph cable off Santiago de Cuba, in 90 fathoms, by Captain Cole of the Telegraph steamer "Investigator," are stunted, sharply striated, the striæ almost ribbon-shaped. The calicles are sunken, often deformed, and sometimes surrounded by shallow open cells, twice as numerous as the septa, producing a resemblance to some of the double-walled palæozoic corals.

Many specimens are deformed by barnacles occupying the end of the branches, which soon become entirely covered by the coral, with the exception of a small opening.

Range from 56 to 262 fathoms, in twenty-seven stations, off Santa Cruz, Saba Bank, Montserrat, Guadeloupe, Dominica, Martinique, St. Vincent, Grenadines, Grenada, and Barbados.

Madracis asperula Edw. & H.

Range from 60 to 248 fathoms, in six stations, off Santa Cruz, St. Kitts, St. Vincent, and Grenada.

Solenosmilia variabilis Dunc.

None of our specimens show the blue coloration noticed by Prof. Duncan in northern specimens.

Range from 120 to 452 fathoms, in six stations, in Old Bahama Channel, off Montserrat, Guadeloupe, St. Lucia, Grenadines, and Barbados.

Lophosmilia rotundifolia Edw. & H.

There is a fine series of specimens of all ages, which positively contradict Duchassaing's opinion that the original specimen of M.-Edwards and Haime was the young of a compound coral, which he has unnecessarily re-named *Oxysmilia rotundifolia.* Occasionally two or three individuals grow in a group, but are not to be called compound for that reason. The lamellar three-lobed columella is rarely seen as regular as in M.-Edwards and Haime's figure ; it usually thickens in the old, and often becomes irregular. The foot thickens very much by additions of exothecal cellular roots arranged in concentric circles, as in Thecocyathus. The dissepiments are few, but rather thick.

Lophosmilia urena Duch. is probably the same.

Range from 42 to 163 fathoms, in eight stations, off Santa Cruz, Montserrat, Dominica, Grenadines, and Barbadoes.

DASMOSMILIA Pourt. nov. gen.

Corallum turbinate, with very thin wall, false palli and columella formed by lobes of the septa ; rudimentary endotheca.

This genus is proposed to receive the two species heretofore named by me *Parasmilia Lymani* and *Parasmilia variegata*, which evidently differ very much from the typical Parasmilia. The figure of one of the septa of *P. Lymani* in my " Deep-Sea Corals," Pl. VI. Fig. 10, represents well the principal generic character.

Dasmosmilia variegata Pourt.

Parasmilia variegata Pourt.
Bathycyathus elegans Studer.

Plate II. Figs. 11 and 12.

The wall measures only 0.003 in thickness in a full-grown specimen; few specimens are therefore obtained entire, and fewer yet are free from deformity from former breakages. Most fragments seem capable of forming new individuals by completing lost parts; sometimes two individuals rise from the septa of one fragment; in that case one of them is most probably a true bud.

In 164 fathoms, off Grenada.

Parasmilia fecunda Lindstr. (Pourt. sp.)

Cœlosmilia fecunda Pourt.
Cœnosmilia arbuscula Pourt.
Blastosmilia Pourtalesi Dunc.
Anomocora fecunda Studer.

From the examination of a large number of specimens it appears conclusively that *Cœlosmilia fecunda* and *Cœnosmilia arbuscula* are but accidental variations of the same species. The *arbuscula* form is the normal one, represented by shorter and more massive corallites, with well-developed columella; the *fecunda* form has grown under circumstances which forced it to elongate beyond measure, and at the same time to form all its parts, such as the wall, the septa, and the columella, thinner and more scanty. The extreme forms are easily distinguished as very different, but there are numberless intermediate ones, often parts of the same stock.

With regard to the apparent budding, numerous alcoholic specimens show that Lindström's remark, that the young do not arise through gemmation, is perfectly correct. There is not a single case where the young grows from a living specimen; the supposed parent has in every instance the appearance of having been dead for some time. It is, however, singular, that in many instances the young are grouped with a certain regularity around, and at a little distance from, an older calicle. If, then, the propagation is by eggs, there remains very little reason for separating this form from Parasmilia proper.

Range from 73 to 450 fathoms, in nineteen stations, off Santa Cruz, Montserrat, Guadeloupe, Dominica, Martinique, St. Vincent, Grenadines, Grenada, and Barbados.

Asterosmilia prolifera Pourt.

Ceratocyathus prolifer Pourt.
Paracyathus arcuatus Lindstr.

Plate II. Figs. 9 and 10.

A careful revision shows that I committed a double error in referring the specimens in question to the genus Ceratocyathus, and in placing the latter

among the Trochosmiliaceæ. The specimens of *Ceratocyathus ornatus* used in comparison are true Caryophylliæ, while my *Ceratocyathus prolifer* is a true Asterosmilia, closely related to *Asterosmilia anomala* Duncan, from the San Domingo miocene. It is one of the very few connecting links between the West Indian tertiary coral fauna and the recent one, while there are so many between the European tertiary and the present West Indian deep-sea fauna.

A specimen with calicular gemmation has three young ones of different ages growing out of its calicle, one of them exceeding the parent considerably in diameter. The latter was still living in the very small part of its calicle left free, and had formed new septa against the outer wall of some of the younger ones. The polyp has a well-developed outer sphincter, which contracts sufficiently to cover entirely the tentacles, and close about two thirds of the calicle.

I may as well remark here that Prof. Duncan's supposition, that the office of the pali is to support an extra circle of tentacles, is not borne out in this species, nor in any other paliferous coral of which I have had the opportunity of examining the polyp. The pali generally show themselves through the membrane of the buccal disk, which they appear to support.

Range from 76 to 94 fathoms, in three stations, off Grenada and Barbados.

Balanophyllia palifera Pourt.

A vertical section shows that the pali are true pali, separated from the septa by a row of perforations. Large specimens have a few septa of the fifth order.

Range from 82 to 164 fathoms, off Guadeloupe, Grenada, and Barbadoes.

Trochopsammia infundibulum Pourt.

A specimen brought up living has the polyp uniformly dark brown, with thick tentacles, slightly different in size, according to their order, and nearly in one circle. There appears to be no muscular circle outside of the tentacles.

Range from 291 fathoms, off Grenada, to 424 fathoms, off St. Vincent.

Stereopsammia? rostrata Pourt.

Amphihelia rostrata Pourt.

This rather abundant coral shows in its younger branches decided Eupsammian characters, the cœnenchyma being perforated in the furrows even at a distance from the calicles; the secondaries are shorter than the tertiaries, which meet in front of them. In old specimens this character becomes less distinct, and the perforations are obliterated by an epithecal growth, which is deposited chiefly on the back part of the branches to a thickness of as much as two centimeters. The striæ are never obliterated by it, but the fine spines disappear gradually, and old calicles and foreign bodies become quite covered by it. The projection from the side of the calicle, on account of which the specific name was given, is very variable, even in the same stock, some calicles

showing but a slight thickening of one of the septa, while in others as many as five septa are swelled out, forming a protuberance equal to the diameter of the calicle.

As the columella is absent or extremely rudimentary, this coral is placed provisorily in the genus Stereopsammia, though it differs considerably from the typical species.

Dendrophyllia profunda Pourt. ought to be placed in the same genus.

Stereopsammia rostrata is one of the largest West-Indian deep-sea corals; some of the stocks when entire must have been an inch thick and a foot high.

Range from 164 to 580 fathoms, in three stations, off Santa Cruz, St. Lucia, and Grenada.

Dendrophyllia Goësi Lindstr.

Like Mr. Lindström's specimens, ours are simple, like Balanophyllia; one of them shows two buds on its sides. As Lindström remarks, it is difficult to draw the line between Balanophyllia and Dendrophyllia. In the case of *Dendrophyllia cornucopia*, for instance, we have in the collection large specimens without any tendency to bud, which if known alone would certainly be classed with Balanophyllia.

From 250 to 400 fathoms, off Havana.

Dendrophyllia alternata Pourt. n. sp.

Plate II. Figs. 3 and 4.

Corallum branching more or less in a plane; calicles on the sides, alternate. Cœnenchyma striated, finely and sharply granulated, feeling rough to the touch. Calicles prominent, and somewhat expanded at the border. Costæ very rough, spines perforated, but not very distinct near the calicle, at a little distance from which they merge into the striæ of the stem. Septa thickened and rough on the edge of the calicle, coarsely granulated on the sides, often bent and warped. Four cycles, six unequal systems, the fourth cycle being unequally developed. The primaries are slightly thicker, and more exsert than the others. Fossa rather deep; columella small, but very compact, and projecting from bottom of fossa, formed of four or five combined parts.

The largest branch is 10 cm. high, 12 mm. in diameter at thickest part; it seems to have been part of a still larger branch. Calicles 5 mm. in diameter.

This species is allied to *D. ramea*, but is smaller, and has no terminal calicles different from the lateral ones.

Range from 150 to 189 fathoms, in three stations, off Guadeloupe, Martinique, and St. Lucia.

Dendrophyllia cornucopia Pourt.

Range from 73 to 400 fathoms, in five stations, off Havana, Grenada, and Barbados.

Bathyactis symmetrica Mos.

Fungia symmetrica Pourt.

The tentacles are rather small, and are arranged, as in the true Fungiæ, at variable distances from the mouth, according to the order of the septa, but as in the latter are very symmetrical; the tentacles are also at regular distances, according to the cycle.

Range from 116 to 400 fathoms, in thirteen stations, off Havana, Santa Cruz, Montserrat, Guadeloupe, Martinique, St. Lucia, Grenadines, Grenada, and Barbados.

Guynia annulata Dunc.

Range from 150 to 357 fathoms, in three stations, off Saba Bank, Montserrat, and Martinique.

Duncania barbadensis Pourt.

I group this and the following species together provisionally, but not under the name of Rugosa, a group which requires revision, and among the characters of which a tetrameral arrangement of the septa cannot be maintained. It is rather singular that no other specimen of Haplophyllia has been obtained in all the dredgings taken in West-Indian waters. It is much to be regretted, as the typical specimen was somewhat deformed.

Range from 103 fathoms, off Barbados, to 191 fathoms, off Martinique.

Anthemiphyllia patera Pourt.

Plate II. Figs. 5 and 6.

The description in my former paper was based on a single specimen. A number of fine ones obtained this year in the same locality renders it necessary to modify it in several points.

The outer surface is covered with a smooth porcellaneous epitheca, without distinct border, concealing the costæ nearly up to the border of the calicle, where they become somewhat prominent, and beset with short spines. There is a coarse spongy columella, with flat fasciculate or oftener foliaceous surface. The interseptal chambers are open down to the bottom, but constricted very much at intervals by a series of stout half floors or shelves projecting from the columella outward. The wall is thick. The transversely flattened spines of the septa are similar to those of *Diaseris crispa;* similar ones are seen also in well-preserved specimens of *Montlivaultia bormidensis.*

I am still in doubt about its affinities; in general appearance it resembles an Antillia, but the absence of a complete endotheca is against placing it in that proximity. It may possibly be related to Discotrochus.

From 250 to 400 fathoms, off Havana.

ANTIPATHARIA.

In determining the Antipatharia of this collection, an attempt was made to use the differences in the shape of the polyps, and in the disposition and form of the spines, to draw characters for a much-needed revision of their classification. It is generally conceded that the division into genera, based mainly on the mode of branching, as established by Milne-Edwards and Haime, is not satisfactory. I have used in former papers the name Antipathes as sole generic designation, and shall continue to do so for the present, until more material is accumulated.

In Plate III. will be found figures, drawn with the camera, of the spines of the West Indian species, and of a few others for comparison. It will be seen that there are at least two different types, — the triangular compressed, and the more cylindrical. The latter are generally more densely set, even assuming sometimes a brush-like appearance, as in *Antipathes humilis.* (Plate III. Figs. 18 and 19.) They are also unequal on the two sides of the pinnules, being longer on the side occupied by the polyps, with a few very much longer ones around the latter. The triangular spines are disposed regularly in a quincuncial order around the pinnules, and in a cleaned specimen nothing indicates the place formerly occupied by the polyps. The only exception to a more or less spiral disposition of the spines with regard to the axis I have found in *Antipathes* (*Cirrhipathes*) *Desbonni*, where the spines are in regular verticils. (Plate III. Fig. 6.) Duchassaing and Michelotti have figured the same arrangement in *Arachnopathes paniculata* D. & M. (non Esper), which I have not seen. In Plate III. Fig. 24, the spines of a very large apparently undescribed species from Mauritius are figured, showing frequently a secondary point, somewhat like shark's teeth.

With regard to the polyps, the drawings herewith presented have the disadvantage of having all been made from alcoholic specimens, in various stages of contraction. Still there are differences from one species to another which cannot be ascribed to that cause. There appears to be a connection between the shape of the polyps and the shape and disposition of the spines. Those species which have triangular spines have polyps with longer tentacles than those with cylindrical spines, with a greater tendency to become regular in shape, though there are some in which the polyp is very oblong in horizontal outline, as in *A. tetrasticha.*

Very long tentacles are found in *A. spiralis.* In very few instances the tentacles are found retracted, as figured by Lacaze Duthiers; in most cases they are simply contracted, and in many species they are probably not retractile at all.

The following species were collected: —

Antipathes (Cirrhipathes) Desbonni Duch. & Mich.

Plate III. Fig. 6.

In former papers I had used this name for a Cirrhipathes bent in a spiral, although the above authors state their species to be straight. In this collection there is a straight form, besides a large number of spiral ones; and as they are specifically quite distinct, I retain the above name for the species more fully described here.

Antipathes growing in clusters, a dozen or more stems from an expanded root, each stem undivided, slender, straight or slightly bent, but not spiral, hollow near the end. Spines small and rather blunt, in regular verticils, of which there are about thirty to a centimeter, each one composed of about twenty spines. On the older parts of the stem the verticils lose somewhat of their regularity, but can always be recognized with a little attention. Vertically the spines are also disposed in straight rows, not winding spirally around the stem. The tips of the stems are membranous and collapsed when dry, being thin and hollow, with the spines already quite distinct. Longest stem, 70 cm.; diameter at base, 1.5 mm.

Only one dry cluster was obtained in station 155, 88 fathoms, off Montserrat.

Antipathes spiralis Pallas.

Plate III. Figs. 5, 25, and 26.

This is the species I had formerly referred to *A. Desbonni* Duch. & Mich. It may be different from Pallas's species, but I have now no means of comparison. Our specimens are all very slender, wound nearly from the base into spirals 10 to 20 cm. in diameter. The spirals are either from right to left, or the reverse, and sometimes change from the one to the other in the same specimen. The spines are short, triangular, compressed, and never in verticils, but in quincunx. The longest specimen is 3.20 m. long, 4 to 5 mm. in diameter at the base.

The polyps are alternately large and small, have very long digitiform tentacles, much longer than have been figured of any Antipathes before. (Plate III. Figs. 25 and 26.) The figure represents them as they are frequently disposed, the larger polyps alone being visible, the smaller ones showing only in the profile view. At other times the tentacles are very much shortened and stiffened, and stand out like those of *A. arborea* figured by Dana.

The cœnosarc on the back part of the branch shows transverse canals more transparent than the rest, in the spaces between successive polyps.

This species is very common, having been obtained in twenty-three stations, in depths ranging from 45 to 878 fathoms, off Havana, Santa Cruz, Montserrat, Martinique, St. Vincent, the Grenadines, Granada, and Barbados.

Antipathes (Rhipidipathes) tristis Duch.

Plate III. Fig. 10.

Of this delicate species there are several good specimens, 3 or 4 inches high; the branches are very slender; anastomoses among them are not plentiful, they are more properly adherences. The spines are sharp, triangular. (Plate III. Fig. 10.) The polyps are small, have short digitiform tentacles, and moderately prominent mouth; the two lower tentacles are sometimes laid around the mouth, as figured in *A. spiralis*.

Range from 45 to 226 fathoms, in eight stations, off Santa Cruz, Montserrat, Martinique, St. Lucia, and Barbados.

Antipathes thyoides Pourt. n. sp.

Plate III. Figs. 17 and 31.

Densely flabellate, but entirely without adherences of the branchlets, which ramify from the sides of the branches without showing any regular pinnate arrangement. The finer branchlets show an apparent succession of swellings, produced by the larger spines surrounding the polyps. The spines are of the cylindrical type, unequal, with a few very long ones about the proximal end of every polyp. (Pl. III. Fig. 17.) The polyps are of the sessile type, with very short tentacles. (Pl. III. Fig. 31.)

The largest specimen spreads 20 cm. in height, and 30 cm. in breadth.

In 124 fathoms, off St. Vincent.

Antipathes picea Pourt. n. sp.

Plate III. Figs. 9 and 29.

Branching, flabellate, the branches with four rows of pinnules, two of which remain generally small and simple, while the two others develop more and give the pinnate appearance to the branches. These larger branchlets are again beset with small pinnules on one side. This is precisely the same arrangement as in *A. tanacetum*, from which it differs by the spines, which are in the latter species about three times as long as broad at the base, while in *A. picea* they are about as high as broad. The polyps are small, with a large spherical buccal knob and flattened tentacles, with slightly incised border; when strongly contracted they appear globular. They are thickly beset with bundles of lasso-cells. On the thicker branches the polyps are rare, and have

distant and rudimentary tentacles ; on the main stem very few buccal knobs are found, and these entirely destitute of tentacles.

Height of specimens 20 to 25 cm.

Station 260, 291 fathoms, off Grenada. Station 286, 7 to 45 fathoms, off Barbados.

Antipathes tanacetum Pourt. n. sp.

Plate III. Fig. 13.

The mode of branching and the spines have been described under the preceding species, and the differences pointed out. This species remains mostly with a simple stem, rarely branching a few times, and has much the appearance of a leaf of tansy or yarrow. On the lower part of the stem the spines become very slender and branching like miniature deer-horns, forming a velvety covering, which becomes filled with grains of sand, sponge spicules, &c. The polyps were badly preserved, but evidently very small.

Most specimens have a parasitic worm, resembling very much, and perhaps identical with, the one which produces the tube in *A. columnaris;* here however, it remains applied to the stem, partly protected by the branchlets, but producing no change in their growth.

Range from 88 to 170 fathoms, in eight stations, off Santa Cruz, Montserrat, Dominica, Martinique, the Grenadines, and Grenada.

Antipathes filix Pourt.

Plate III. Figs. 15 and 16.

My original description of this species was based on simple and younger stocks; it, however, branches in a subflabellate manner, spreading 30 to 40 cm., more in breadth than height, and assuming then a general appearance with *A. myriophylla* of the East Indies, with which I have confounded it when in this state (Bull. Mus. Comp. Zoöl., Vol. V. No. 9). It differs from it greatly in the arrangement of the pinnules and spines. The long spines surrounding the polyps are beset with little knobs at the end, giving them a rugose appearance. In *A. myriophylla* (Pl. III. Fig. 23) the spines are all equal.

The polyps are small and inconspicuous, and of the type of those of *A. humilis* (Pl. III. Fig. 32).

The differences between this species and *A. abietina* are not great, the spines and polyps presenting no particular differences. The latter species may be distinguished, if it is not considered a mere variety, by its greater stiffness, and by being regularly pinnate instead of having pinnules in every direction.

Range from 76 to 287 fathoms, in twenty stations, off Montserrat, Martinique, Dominica, Guadeloupe, St. Vincent, the Grenadines, and Barbados.

Antipathes eupteridea Lamx.

Plate III. Fig. 11.

The very scanty description of this species, the type of which came from Martinique, leaves it a little doubtful if our specimen can be referred to it. Lamouroux compared his specimen to a peacock's feather. Ours is branching, resembling very much some of the larger *Plumularidæ*, — *Cladocarpa paradisea* Allm., for instance. The specimen, the main stem of which was dead at the top, must have been about 40 to 50 cm. high. The pinnules are 40 mm. long. The spines are nearly cylindrical, rather dense, subequal, very little longer about the polyps. The polyps are very small and sessile.

Station 203, 96 fathoms, off Martinique.

Antipathes salix Pourt. n. sp.

Plate III. Fig. 8.

Irregularly branching, with long slender pinnules, not disposed in any particular order, the whole appearing somewhat like a miniature weeping-willow. The spines are equal, long triangular, somewhat hooked upward, rather close set. On the larger branches they form longitudinal rows, more or less regular. The polyps are very small and inconspicuous, of the sessile type.

It resembles somewhat *Arachnopathes paniculata* Duch. & Mich. (non Esper.), but is more flexuous, has no coalescent branches, and the spines are not in verticils.

Station 171, 183 fathoms, off Guadeloupe.

Antipathes rigida Pourt. n. sp.

Plate III. Fig. 12.

A small specimen, differing from the preceding only in being stiffer, with thicker pinnules and occasional coalescences of branches. The spines are very much like those of the preceding species, only not quite as densely set. The polyps are of the same type.

Station 273, 103 fathoms, off Barbados.

Antipathes columnaris Duch.

Plate III. Fig. 3.

The spines are very small, triangular, and blunt, somewhat longer at the tip of the pinnules. The polyps are rather abundant on the network forming the tube for the parasitic worm.

Range from 73 to 861 fathoms, in sixteen stations, off Guadeloupe, Martinique, Dominica, Virgin Gorda, St. Lucia, St. Vincent, the Grenadines, and Barbados.

Antipathes humilis Pourt.

Plate III. Figs. 18, 19, and 32.

The most densely spinous species which has come under my observation.

Range from 76 to 262 fathoms, in four stations, off Montserrat, Grenada, St. Vincent, and Barbados.

There are several species described by Duchassaing and Michelotti, and by the former alone in his *Revue des Zoophytes et Spongiaires des Antilles*, Paris, 1870, which are too briefly characterized for identification. The large species which I had formerly referred to *A. dissecta* D. & M. is *A. glaberrima* Esper.

EXPLANATION OF THE PLATES.

PLATE I.

Fig. 1. *Deltocyathus italicus*, showing passage from variety *Agassizii* to variety *calcar*.
Fig. 2. *Deltocyathus italicus*, variety α, *Agassizii*, section magnified.
Fig. 3. Costæ of the same, magnified.
Fig. 4. *Deltocyathus italicus*, variety β, section magnified.
Fig. 5. " " " γ, *calcar*, section magnified.
Fig. 6. " " " δ, section magnified.
Fig. 7. " " " δ, costæ magnified.
Fig. 8. " " " δ, calicle magnified.
Fig. 9. *Paracyathus laxus*, profile, nat. size.
Fig. 10. " " calicle, "
Fig. 11. " " section, "
Fig. 12. *Caryophyllia communis*, variety *costata*, nat. size.
Fig. 13. " " " " calicle, nat. size.
Fig. 14. *Desmophyllum Riisei*, nat. size.
Fig. 15. *Stenocyathus vermiformis*, portion decalcified, magnified.
Fig. 16. " " " two segments separated, to show interrupted connections at the centre.

PLATE II.

Fig. 1. *Stephanotrochus diadema*, specimen with the polyp contracted, in alcohol.
Fig. 2. *Stephanocyathus variabilis*, " " " "
Fig. 3. *Dendrophyllia alternata*, nat. size.
Fig. 4. " " calicle magnified.
Fig. 5. *Anthemiphyllia patera*, section "
Fig. 6. " " septa "
Fig. 7. *Paracyathus flos*, profile, nat. size.
Fig. 8. " " calicle, "
Fig. 9. *Asterosmilia prolifera*, calicle, nat. size.
Fig. 10. " " profile, "
Fig. 11. *Dasmosmilia variegata*, profile, nat. size.
Fig. 12. " " calicle, "
Fig. 13. *Flabellum Moseleyi*, profile, nat. size.
Fig. 14. " " calicle, "

PLATE III.

Fig. 1.	Pinnule of	*Antipathes*	*tetrasticha,*	magnified	10 diameters.	
Fig. 2.	"	"	*glaberrima,*	"	"	
Fig. 3.	"	"	*columnaris,*	"	"	
Fig. 4.	"	"	*lenta,*	"	"	
Fig. 5.	Stem of	"	*spiralis,*	"	"	
Fig. 6.	"	"	*Desbonni,*	"	"	
Fig. 7.	Membranous tip of	*Antipathes*	*Desbonni,*	"	"	
Fig. 8.	Pinnule of	*Antipathes*	*salix,*	"	"	
Fig. 9.	"	"	*picea,*	"	"	
Fig. 10.	"	"	*tristis,*	"	"	
Fig. 11.	"	"	*cupteridea,*	"	"	
Fig. 12.	"	"	*rigida,*	"	"	
Fig. 13.	"	"	*tanacetum,*	"	"	
Fig. 14.	"	"	*abietina,*	"	"	
Fig. 15.	"	"	*filix,*	"	"	
Fig. 16.	Spine of	"	*filix,*	magnified	230 diameters.	
Fig. 17.	Pinnule of	"	*thyoides,*	magnified	10 diameters.	
Fig. 18.	"	"	*humilis,*	"	"	upper view.
Fig. 19.	"	"	"	"	"	lower view.
Fig. 20.	"	"	*Fernandezii,*	"	"	
Fig. 21.	"	"	*arborea,*	"	"	
Fig. 22.	"	"	*reticulata,*	"	"	
Fig. 23.	"	"	*myriophylla,*	"	"	

Fig. 24. Pinnules of *Antipathes*, sp. from Mauritius, magnified 10 diameters.

Fig. 25. Side view of contracted polyps of *Antipathes spiralis*, showing smaller polyps concealed under the arms of the larger, magnified 10 diameters.

Fig. 26. Upper view of contracted polyps of *Antipathes spiralis*, the small polyps showing but one or two tentacles; the tentacles of the large one in the middle are now contracted.

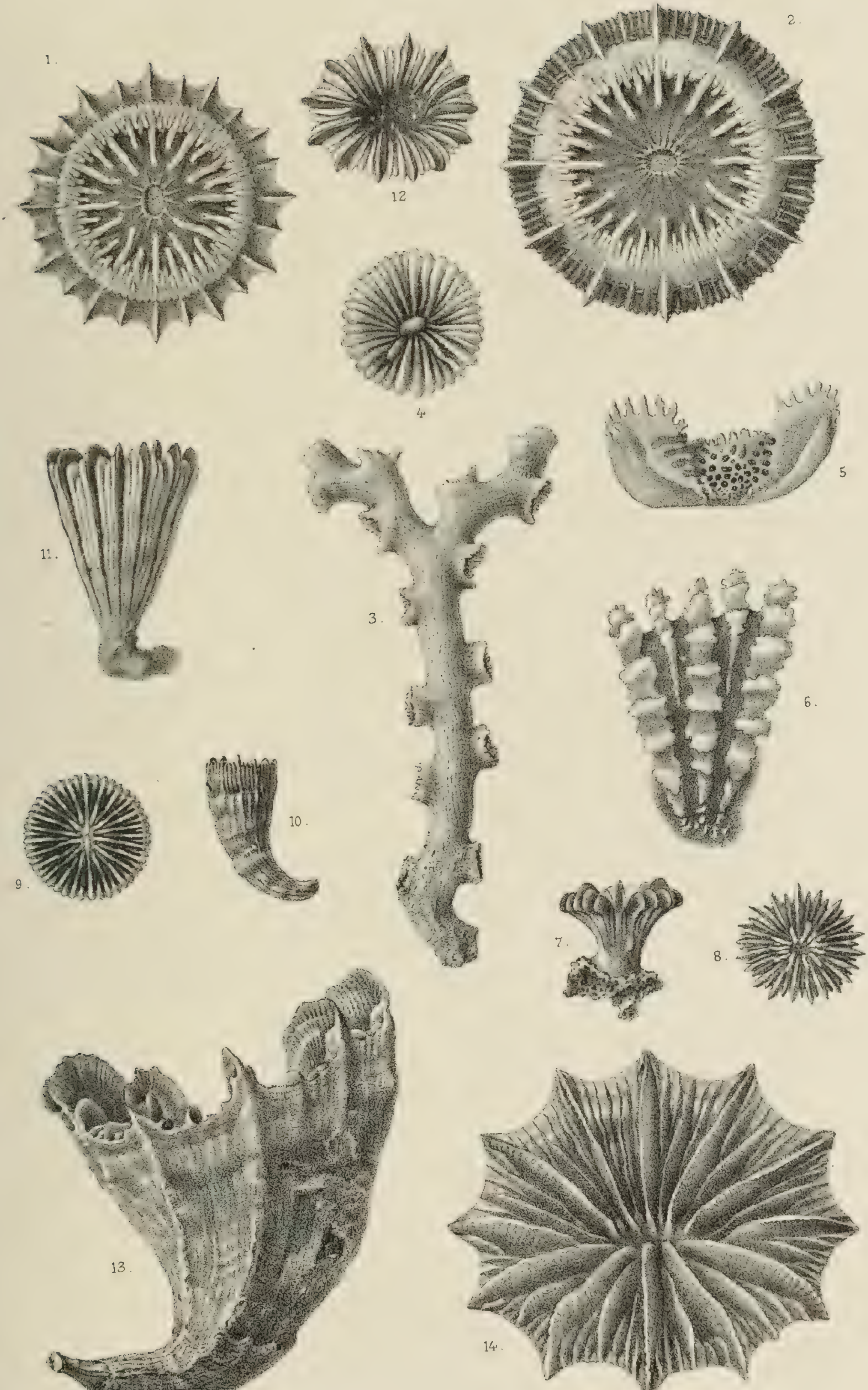
Fig. 27.	Polyps of	*Antipathes*	*lenta,*	magnified	10 diameters.
Fig. 28.	"	"	*glaberrima,*	"	"
Fig. 29.	"	"	*picea,*	"	"
Fig. 30.	"	"	*tetrasticha,*	"	"
Fig. 31.	"	"	*thyoides,*	"	"
Fig. 32.	"	"	*humilis,*	"	"

CAMBRIDGE, February 6, 1880.

Pl. I

A. Meisel, Lith

Pl II



A. Meisel, Lith

Pl. III

A. Meisel, Lith

No. 5.—*The Ethmoid Bone in the Bats.* By Harrison Allen, M. D.

A comparison of the ethmoid bones of the bats, upon which I have been of late engaged, has resulted in defining some interesting points in the anatomy of the organ of smelling in these animals. Awaiting opportunity for framing more elaborate descriptions, I propose formulating an account of the peculiar appearances of the ethmoid in the various families. I may here state, that, in every example I have examined, the detail in the arrangement of the scrolls of the ethmoid bone has yielded characters by which the genera and even the species can be readily determined.

The genera examined are the following: *Pteropus, Epomophorus, Rhinolophus, Phyllorhina, Megaderma, Nycteris, Antrozous, Plecotus, Corinorhinus, Vesperugo, Vesperus, Scotophilus, Atalapha, Vespertilio, Natalus, Miniopterus, Emballonura, Taphozous, Noctilio, Molossus, Nyctinomus, Chilonycteris, Mormoops, Macrotus, Vampyrus, Schizostoma, Phyllostoma, Carollia, Glossophaga, Artibeus, Vampyrops, Stenoderma, Chiroderma, Sturnira, Brachyphylla, Centurio*, and *Desmodus.*

The identifications of Dobson (Catalogue of the Chiroptera Br. Mus., 1878) have been accepted in framing the above list.

In all the genera examined, the ethmoid bone is composed of a vertical lamella projected from the cribriform plate, to which in most instances there is appended an outer (lateral) horizontal scroll.

(1.) In its simplest form, the vertical plate bears upon its median surface one or more rudimental scrolls. Examples of this variety are seen in the *Nycteridæ*. In *Nycteris, Rhinolophus, Phyllorhina*, and *Megaderma spasma*, the rudimental scrolls are horizontal; but in *Megaderma frons* they are vertical. The outer (lateral) scroll, which is present in *Nycteris* and *Phyllorhina*, tends to be directed inward.

(2.) In the next degree of complexity met with, the vertical lamella resembles the foregoing, but possesses a small lateral scroll, which arises independently from the cribriform plate. The vertical plate retains upon its median aspect two vertical rudimental scrolls. Example, the genus *Emballonura.*

(3.) In the third degree of complexity the vertical plate is revolute anteriorly, and (as seen from above) is sub-triangular or cylindroid in form. It retains upon its median surface two supplemental horizontal or oblique scrolls. The outer (lateral) scroll is present.

The vertical plate may project well in advance of all the other parts, or may be but slightly longer than they. No union exists between the outer (lateral) scroll and the vertical plate of the frontal bone in the orbit. Examples, *Vespertilionidæ*, the genus *Molossus*, its congeners, and *Noctilio*. *Natalus* is remarkable for lacking the outer (lateral) scroll.

In *Molossus*, *Nyctinomus*, and *Noctilio* the vertical plate projects scarcely at all in advance of the median supplemental scrolls, and never appears on the median surface below the level of the scrolls. In *Vespertilionidæ* it forms a conspicuous tapering process. It is seen below the plane of the supplemental scrolls in *Atalapha noveboracensis* and *Vesperus noctivagans*.

(4.) The vertical plate is short and ends abruptly anteriorly. It is visible beneath the supplemental scrolls on the median surface. The outer (lateral) scroll is as long as the vertical, and is united to the vertical orbital plate of the frontal bone. Example, the genus *Taphozous*.

(5.) The vertical plate is produced in advance of the position of the supplemental scrolls, as in the last-named group, but is compressed from side to side as seen from above, and is not revolute. It bears upon its median aspect posteriorly a lobule. The supplementary scrolls in general appearance are much as in the *Vespertilionidæ*. The lateral scroll is cylindroid. Examples, the *Phyllostomidæ*.

In *Desmodus*, the lobule upon the anterior portion of the vertical plate is relatively large.

(6.) The vertical plate is projected far in advance of the supplemental scrolls, which are horizontal in position and four in number. The lateral scroll is more or less adherent to the vertical plate, or by its outer border to the frontal bone. Examples, the *Pteropidæ*.

The *Pteropidæ*, *Nycteridæ*, and some *Phyllostomidæ* have a horizontal septum passing transversely from the under free edge of the vertical plate (as it lies beneath the lowest median supplemental scroll) to the nasal septum. The olfactory surface in such forms is thus withdrawn from the respiratory currents, since no direct outlet exists at the posterior nares.

The above descriptions have been drawn, for the most part, from specimens in the fine collection of the Museum of Comparative Zoölogy, Cambridge, Mass.

February, 1880.

No. 6. — *On certain Species of Chelonioidæ.* *By* Samuel Garman.

In this notice three species of Sea Turtles are mentioned, of which two are supposed to be new.

About three years ago, Richard M. Kemp, of Florida, directed my attention to a peculiar Turtle, commonly called the "Bastard," found in the Gulf of Mexico, and said to be a cross between the Green and Loggerhead, *Chelonia mydas* and *Thalassochelys caouana.* At a later date he secured for the Museum a pair of fine specimens, which furnish the material for a description given below. In consideration of the great interest Mr. Kemp takes in matters pertaining to natural history, it is most appropriate that the species he has been the means of bringing into notice should bear his name.

There is considerable likelihood that the other species, of which descriptions are given, have heretofore been considered as one, *Chelonia virgata.* If this has been the case, a very slight comparison of the characters assigned will convince any one of the necessity of separation. Of the various names that have been applied by different authors to *C. virgata,* none can be said with certainty to belong to the flat, broad species which has probably been associated with it. Consequently, it is thought better to apply a name not previously employed in connection with either of them, thus avoiding confusion, rather than to make use of a synonyme concerning which there will always exist more or less doubt.

Thalassochelys Kempii sp. nov.

Body depressed, short, broad, subcircular, with a slight concavity over the lateral marginal plates of the carapace, and without the prominent rounded hump on the vertebral series over the pelvis or shoulder girdle, as in *T. caouana.* Head intermediate in size between that of *T. caouana* and that of *Chelonia mydas,* crown slightly convex. There is a shallow depression from the eye forward. Looking from above, the outline of the face is much more convex than in either of the species cited. A low, broad, rounded ridge extends from the nostrils to the point on the end of the beak. The lateral outline of the jaws

is very convex forward. Upper jaw without serrations, lower outline forming a sigmoid curve, convex posteriorly, and concave near the extremity, where it suddenly descends to the sharp point at the symphysis. The greatest convexity occurs at a point below and in front of the eye. Lower jaw strong, without serrations, upper edge concave, curving upward in a point on the symphysis. Frontals, two pairs. Vertical small, narrow, hexangular. Two supraorbitals on each side. Interparietal large, broad, surrounded by thirteen plates (9–13). Postorbitals, three, upper small, lower narrow, elongate. Carapace with little or no indication of a hump on the first or ultimate vertebral plates, outline slightly straightened over the hind legs, indented over neck and arms, with five shields in each series of costals and the vertebral. Anterior vertebral shield short and narrow, second to fourth narrow and long, posterior longer and wider. First pair of costals small. Marginal plates, twenty-seven, anterior very narrow, becoming wide on the flanks from the fourth. From the middle of the body back the marginal shields are subequal, excepting the caudal pair, which are wider, but without being produced beyond the general outline. Eight or ten of the posterior marginal bones of the skeleton are joined by suture to the broad costals, making for the hinder half of the carapace nearly solid bone. Paddles medium, each with two nails, anterior long and narrow, posterior short and broad, margins indented between the digits.

In one specimen the width and length are equal, twenty-six inches; in the other, the width is twenty-nine inches, while the length is only twenty-eight. Both are quite aged, as is shown by the ossification of skull and carapace, and by the worn appearance of jaws and scales.

Distinguished from *T. caouana* by the short, round body, low humps, marginal plates, narrowness of head across occiput, and swollen jaws; from *T. olivacea* by shape of head, swollen jaw, and plates of the carapace. The compression of the anterior portion of the head of *T. olivacea* at once separates the species.

"The Bastard Turtle are common. We know that they come on the beach to lay in the months of December, January, and February, but cannot tell how often, or how many eggs they lay at a time. They can be secured quite readily, but are not sought for. Hawksbill, Loggerhead, and Green Turtle lay in April, May, and June." (Kemp.)

Some of the characters by which this turtle is distinguished from *caouana* and *olivacea* are of more than specific importance, — namely, shape of head and body, and skeletal peculiarities. According them a subgeneric value, the habitat suggests the name *Colpochelys*, from κόλπος, a gulf. This will give to this species the name *Colpochelys Kempii*, Kemp's Gulf Turtle.

Chelonia depressa sp. nov.

Young. — Body a broad oval; head large, rounded posteriorly, occiput convex, flattened between and compressed in front of the eyes. Jaws not serrate (in very young), upper with a shallow notch in front, lower with a sharp

curved prominence at the symphysis. Carapace broad, arch comparatively low, with three low ridges, slightly concave near the margin. Paddles broad, rounded on the margins.

Adult. — Body broad, depressed, subelliptical, broadest near or behind the middle, concave near the lateral margins, flattened over the second to the fourth vertebral plates; head larger and broader than that of *C. mydas* or *C. virgata*, broad posteriorly, convex on the occiput, flattened between and compressed in front of the eyes. Upper jaw not serrated, outline nearly straight, with the notch at the symphysis almost obliterated, vertically grooved on the inner face. Lower jaw serrated, bearing a curved fang-like prominence on the symphysis. Carapace broad and spreading posteriorly, arch very low. Paddles comparatively small, anterior narrow and pointed, posterior short, truncate, indented between the digits. One pair of elongate frontals. Vertical small, short, broad, pentagonal, acute-angled in front. Supraocular large, broad. Interparietal broader than long, surrounded by seven plates, vertical, supraoculars, parietals, and occipitals. Postorbitals four (3–4), lower large. Plates of carapace not imbricate, smooth in young and adult, costal series four each, five vertebrals, and twenty-five marginals. Sternal plates thirteen, in two series of six each, preceded by a small triangular plate at the neck. Lateral plates of plastron four on each side, preceded by a pair of small, and these again by several smaller brachials. The specimens described are from the East Indies and North Australia. Applying the line to the shell the Australian specimen measures in length $36\frac{1}{2}$ inches, and in width 30 inches; its height is 9 inches. A specimen of *C. mydas* has a length of $39\frac{3}{4}$ inches, a width of $34\frac{1}{2}$ inches, and a height of 11 inches.

C. depressa differs much from the species described by Dumeril and Bibron as *C. virgata.* It is less truncated and more deeply indented in front than either of the other species of the genus. A transverse section across the middle of the body is not what would be called roof-shaped, but more of the shape of a bow of considerable curvature, a portion of the middle of which is straight, and of which the extremities are sharply turned upward. The sides are not strongly arched, and the cross-section of a large specimen could not be described as forming an open angle. The broadness of the head, the marked difference in shape from that of *C. mydas,* and the concavity near the lateral margins, could not have escaped the notice of the authors of the *Erpétologie Générale,* if there were specimens of this species at hand. Their description applies either to the species renamed by Dumeril and Bocourt *C. Agassizii,* or to one much more closely allied to it than that described above. If the separation of *C. Agassizii* from *C. virgata* of authors is right, there exists a third species of *Chelonia* in the Northwestern Pacific and the northern part of the Indian Ocean. The specimens from which the description in the *Erp. Gén.* was taken were said to be from Teneriffe, Rio Janeiro, Cape of Good Hope, New York, and the Indian Ocean, which distribution can leave little doubt that they were of more than one species.

Chelonia Agassizii Duméril & Bocourt, 1870.

? *Chelonia virgata* Schweigger, 1814.
Chelonia virgata Agassiz, 1857.

Carapace subcordiform, considerably arched, narrow posteriorly; margin with a shallow indentation over neck and each arm, and a deeper one over each leg. Head moderate, about the size and shape of that of *C. mydas*, more compressed and pointed in front of the eyes than that of *C. depressa*. Upper jaw not serrate on the edge, with grooves on the inner face corresponding to the teeth on the lower, with a slight notch in front. Lower jaw serrated on the edge, bearing a prominent curved point on the symphysis. Serration of jaw not apparent in very young specimens. Frontal plates one pair, sometimes subdivided. Vertical small, narrow. One supraocular on each side. Interparietal moderate, surrounded by seven plates; supraoculars and vertical in front, and a pair of large plates behind on the occiput. Postoculars four. Central plates of carapace thirteen, vertebral series five, anterior and posterior wider, posterior costals and vertebrals sometimes divided; marginals twenty-five, posterior sometimes fused. The anterior and posterior plates of back are rather suddenly bent downward near the margin. The tail of the male is longer; it appears that the pointed extremity of the carapace is also more elongate in this sex.

Specimens described from the eastern portion of the tropical Pacific.

Cambridge, March, 1880.

No. 7. — *Contributions to a Knowledge of the Tubular Jelly-fishes.*
By J. Walter Fewkes.

I. The Development of the Tentacular Knob of Physophora hydrostatica.

The anatomy of those animals known to the zoölogist as the Siphonophoræ, or tubular Jelly-fishes, has been carefully studied, and minutely described. I present certain points in which my observations or conclusions differ from those of other naturalists. I have also discussed at length the limits and synonymy of the genus *Halistemma*, since I think it embraces animals with generic differences, and I conclude with a brief mention of North American Siphonophoræ and Velellidæ, adding three genera to those already described for our coasts. The development of the structures which have received the name of tentacular knobs has a certain interest, particularly the different stages in growth of that perhaps most complicated of all, the knob of *Physophora*. The development of these structures long since attracted attention, and Claus * twenty years ago (1860) published a description, with figures, of the younger stages of the knob in *Physophora hydrostatica*. Keferstein and Ehlers, to whom science owes so many discoveries in regard to these Jelly-fishes, followed this work with certain corrections and additions of the most important character. Their investigations were made upon a species of *Physophora* called *P. Philippi*, identical with or only distinguished from *P. hydrostatica* by the possession of lateral appendages to the external walls of the knob. The account which they give in most particulars applies also to *P. hydrostatica*, which has furnished me the material for my studies of the developmental history of the tentacular knob in this genus.

The growth of the knob of *Physophora*, although quite simple, is more complicated than that of any other Siphonophore. I have only, however, considered it necessary to figure a few stages assumed in this growth, illustrating the peculiar asymmetrical form of the involucrum, and the embryonic appendages to the sacculus, which are provisional in

* Ueber Physophora hydrostatica nebst Bemerkungen über andere Siphonophoren.

their nature, and give the early condition of the knob a likeness to that of certain other genera of Physophoridæ.

The knob of *Physophora hydrostatica* originates, like that of other tubular Jelly-fishes, as a simple bud, hardly distinguishable from the earlier condition of all structures in the Siphonophores. In its place of origin it resembles the genus *Agalma*, for it forms on the ciliated base of the feeding polyp, and is in fact a proliferation of the walls cf that part. Whether all Physophore knobs originate from the same relative position is an open question. In *Rhizophysa filiformis* we have several of the polypites nearest the float with naked tentacles, from which the knobs bud, never arising from the base of the polypites. Of course these undeveloped appendages in the singular *Rhizophysa* may be looked upon as tasters,* a supposition hardly probable; or it is also possible that they correspond with somewhat similar structures between the nectocalyces of *Apolemia uvaria.* In the well-known genus last mentioned the polyp-like parts between the swimming-bells appear to have no filaments like those found on the tasters of other Siphonophores. The taster-like bodies near the float of *Rhizophysa* are undeveloped feeding polyps.

In the very earliest stages the Physophora knob is composed of layers which are apparently two in number. The differentiation of other layers takes place later in the course of the development. At first we find only ectoderm and entoderm in the walls of the knob. This simple bud elongates into a flask-shaped body, at the base of which the cavity becomes enlarged, imparting to this region a more or less spherical shape. (Pl. I. fig. 2.) From the ectodermic wall of the enlargement thus formed arises the involucrum. An examination of this region, even at an early period (Pl. I. fig. 3), shows that a differentiation has begun, and that the ectoderm has divided into two layers, one of which appears as a collar

* The word *taster*, to designate peculiar structures among the Siphonophores, is perfectly applicable in the case of *Physophora.* In other genera the designation "Saftbehälter" may seem better; but here in *Physophora* their function seems different from that of the same part in most of these animals. The filamentary appendage to the taster in Physophora, although very easy to see, has been overlooked by several naturalists. (See Kölliker, *Schwimmpolypen von Messina.* Vogt, *Siphonophores de Nice.* Leuckart, *Siphonophoren von Nizza*, p. 106.) According to Keferstein and Ehlers (*l. c.*, p. 31), these appendages to the taster were discovered by Philippi, but were omitted in the descriptions by naturalists who followed him until the investigations of Sars. In his *Anatomy of Physophora*, Claus (1860) speaks of them (p. 17), but has no representation of the filament in his figure of the genus. (Claus, *Ueber Physophora hydrostatica nebst Bemerkungen über andere Siphonophoren*, Zeitsch. f. Wiss. Zool., Bd. X. p. 1, fig. 1. Philippi, in Müller's *Arch. f. Anat. u. Physiol.*, p. 61, Taf. 5, fig. 4. Sars, in Middelhavet's *Littoral Fauna*, p. 4.)

around the base of the knob. If we watch the growth of this collar, which is the outer differentiated layer of the ectoderm, it will be found to gradually grow down around the sacculus until it has almost completely enclosed it, leaving, however, an opening at the distal pole of the knob, through which the end of the sacculus, or certain appendages to the extremity of this organ, project. (Pl. I. figs. 4, 5.)

Meanwhile, the sacculus has passed through certain changes, the most important of which is a coiling up of itself within the envelope of the involucrum, and the formation at its extremity, where it projects through the opening of the involucrum, of certain appendages of a provisional nature. The earliest condition of the sacculus is simply the terminal transparent part of the flask-shaped body already mentioned. It is now a complicated organ armed with lasso cells, and with its walls highly colored. The provisional structures at the distal end of the sacculus (Pl. I. figs. 4, 5, 6, 8) are mentioned and figured by most of those who have studied the young knob of *Physophora.* They have been seen in both species, but do not appear to exist in the fully developed form of the knob, either in *P. hydrostatica* or *P. Philippi.* The accompanying growth of another part of the young knob is destined to change materially the appearance of the whole, as well as the relative development of the parts. This change takes place contemporaneously with the enclosure of the sacculus by the involucrum, and the appearance of those provisional terminal filaments which I have already mentioned. The alterations of form to which I refer are as follows. The proximal or basal part of the spherical-shaped expansion of the young knob enlarges on one side, and in such a manner that the knob as a whole assumes an asymmetrical shape. (Pl. I. fig. 4.) This want of symmetry is brought about by an unequal growth in the two sides of the basal part of the knob itself. In a still more developed stage of the same structure the inequality in growth has gone still further, and the enlargement lengthens and extends along the side of the sacculus, now coiled on itself, carrying with it the former place of attachment of the sacculus, which is to be found at the opposite pole from its former junction. (Pl. I. figs. 5, 6.)

Meanwhile the knob is approaching its fully-grown form, and the terminal filaments become absorbed; the opening at the distal pole of the involucrum closes or is very much reduced in size, and the enlargement in the spherical cavity, which earlier gave the asymmetrical form to the whole knob, appears as a simple tube following down along the side of the knob from the pedicel to the place of attachment of the sacculus, at

the opposite end from its original junction. In the structure formed by these changes we have the fully-grown condition of the complicated knob of this Jelly-fish. (Pl. I. fig. 7.)

The resemblance of certain of the earlier stages in the growth of this organ, or individual if one so designates it, to the adult in a different genus is very great. *Athorybia* has a tentacular knob with many points of resemblance to the undeveloped forms which have just been described. The figures of the knob of this genus, as given by Gegenbaur, Kölliker, and Huxley, show a close likeness to the younger stages of the knob in *Physophora.*

While emphasizing this asymmetrical growth of the knob of the young *Physophora*, and suggesting a likeness to the same structure in the genus *Athorybia*, I recall the figures of the knob in the younger stages of an *Agalma*, called by Leuckart *Agalma clavatum.* As Claus suggests, this species is probably the young of *Agalma Sarsii.* Leuckart's figures of *A. clavatum* show a knob which assumes a similar asymmetrical shape to that which exists in the knob of *Athorybia.* This naturalist* has already made the comparison of a tentacular knob of *A. clavatum* with the same structure in *Athorybia.* The comparison seems to me a good one, and does not prevent a comparison of both to the undeveloped tentacular knob of *Physophora hydrostatica.* A likeness is further indicated by the existence in each genus of terminal filaments on the sacculus, provisional to be sure in *Physophora*, but none the less definitely pointing out the relation of the structures under consideration.†

II. The Mantle-Tubes of Apolemia uvaria and Gleba hippopus.

A wish to find out the homology of the somatocyst of the Calycophores led me to a study of the chymiferous tubes of the swimming-bells throughout the tubular Jelly-fishes. Especially in *Apolemia* and *Gleba*, from their aberrant forms, I hoped to find some facts bearing on the solution of this question; and when I came to see the former of these genera for the first time, my thoughts were turned to the question of its mantle-tubes. This genus, in many respects allied to the Calycophoridæ, is a true Physophorid; yet, in the published description of its nectocalyces, I find no mention of any structure which, I think, can be truly known as the

* Siphonophoren von Nizza, Pl. XIII. Fig. 5, p. 91.

† I find these structures in *hydrostatica* more leaf-like than they are represented in Keferstein and Ehlers's plate of *Physophora Philippi.*

mantle vessel. The four radial tubes of the bell, and the appendages to the lateral pair, have been well figured and described. Leuckart seems to liken rudimentary offshoots of the lateral vessels to mantle-tubes. I do not think these offshoots more than very distantly comparable with that special pair of vessels, which arises from a tube medially placed in the bell, connecting the junction of the radial system with the stem cavity of the animal. Such mantle-tubes, for instance, as are to be found in *Agalma*, *Gleba*, or other genera, do not seem to have been observed in the nectocalyx of *Apolemia*. I think, however, that I have found in the bell of *Apolemia* a structure homologous to the mantle-tubes in the Physophoridæ, and represented in the Calycophore by the somatocyst.

The mantle-tubes in *Apolemia* are difficult to make out, but seem to differ only in their size from those in *Gleba*. Radial tubes in these two genera, however, differ very greatly; for while in the one they reach a development hardly equalled among Siphonophores, in *Gleba*, where the cavity of the bell is very shallow, and the nectocalyx itself is more of a bract than a swimming-bell, the chymiferous tubes have a minimum development. So rigid is the nectocalyx of *Gleba* that the walls admit of little motion, and most of the propulsion is done similarly to that of *Circe* and other Trachynemidæ, by a movement of the velum, a crescentic-formed vail surrounding the opening into the shallow bell cavity. As a consequence, the radial system is quite diminutive in size. Nowhere among Siphonophores better than in the genus *Gleba* do we find a nectocalyx (Pl. III. Figs. 4, 5), when fully grown, so closely resembling a bract, and it seems to me that a better proof of the homology of the central tube of the bract or covering scale with the mantle vessel of a nectocalyx could hardly be desired.

Apolemia has a float and a true Physophorous nectocalyx,* while *Gleba* has no float, and is radically different from the Calycophoridæ, although its multiplicity of nectocalyces is a true characteristic of the Physophoridæ. Therefore I think that the Hippopodidæ should make one of the three great groups into which the Siphonophoræ may be divided, and be considered an equal division with the Physophoridæ and Calycophoridæ.

* I figure (Pl. I. Fig. 1) a fragment of an *Apolemia*, without nectocalyx or float. I have already published a representation of the nectocalyx of *Apolemia*. Proc. Bost. Soc. Nat. Hist., Vol. XX.

III. The Tubes in the larger Nectocalyx of Abyla pentagona.

The best description which I have found of the course of the chymiferous tubes of *Abyla pentagona* is by Gegenbaur.* At the regular meeting of the Boston Society of Natural History, on November 5, 1879, I pointed out the existence in *Abyla* of a supplementary tube, which takes an origin from the junction of one of the radial vessels with the circumvelar tube, and extends diagonally across one quadrant of the bell, ending in an enlargement of a peculiar kind. I also indicated the difficulties which present themselves to a determination of an homology between the chymiferous tubes in *Abyla* and other nectocalyx-bearing Siphonophores, on account of these supplementary tubes. The bilateral symmetry shown quite well in the swimming-bells of other Calycophoridæ, as *Epibulia, Diphyes,* and *Praya,* in the Hippopodidæ, and in *Agalma, Agalmopsis, Halistemma, Apolemia,* and *Physophora* of the Physophoridæ, does not appear in the different spheromeres of *Abyla.* In all cases except *Abyla,* bilateral symmetry, as referred to a plane passing through two opposite chymiferous tubes of the bell, and the ventral line of the stem, is very easy to make out. The want of symmetry in *Abyla* is the result of a covering in of the "Längskanal" by a growth from one of the bounding ridges of the bell. A like covering of the canal is to be seen in *Monophyes,* where the nectocalyx is hemispherical, with none of those marked elevations and projecting points continued beyond the opening of the bell which are so prominent in *Abyla,* and to which it owes both of the specific names *pentagona* and *trigona.* I have noticed no variation from a normal arrangement of the chymiferous tubes in *Monophyes.* (Pl. III. fig. 6.)

IV. On Halistemma, Agalma, and Agalmopsis.

The adoption of the generic name *Halistemma* has now become almost universal, and seems necessary for a proper understanding of the genera of Siphonophores, about which there has existed considerable confusion. The following animals have, I think, been erroneously placed in this genus; viz. *Halistemma tergestinum,* Claus, and *Halistemma carum,* Haeckel. Huxley, in "Oceanic Hydrozoa," proposed the name for certain forms of tubular Jelly-fishes, with elongated axes, biserial rows of swimming-bells, and naked tentacular knobs with a single terminal filament. The genus *Agalma,* by his classification, was to include those the

* Neue Beiträge zur Näheren Kenntniss der Siphonophoren. Nova Acta Carol., Vol. XXVII., 1860, pp. 349-356.

tentacular knobs of which had two lateral terminal filaments, while *Stephanomia* had but a single filament of this kind, although the last two genera have a biserial row of nectocalyces and an involucrum.

There are certain other characteristics of this genus which are not so well marked as those already given by Huxley. I refer to the character of the tentacles, and more especially to the position of the sexual organs. Tentacles such as we find in *Agalma* do not seem to exist in the genus *Halistemma*, but the tentacular knobs have very long pedicels, longer than in other Physophoridæ, which allow the knob to project so far beyond the covering scale as to resemble tentacles very closely. According to some observers true tentacles do exist in the genus Halistemma. For instance, Leuckart says that Kölliker missed the true tentacle, and mistook the pedicel of the knob for a tentacle itself.* Kölliker's figure of *Agalmopsis punctata*, which is the same thing as *Halistemma rubrum*, shows the absence of the tentacles very plainly. My observations on the tentacle agree with Kölliker's, yet his figure of the animal is not complete, in that he failed to represent the sexual system. The female sexual organs I shall later describe. (Pl. I. figs. 3 – 5.) Leuckart* figures a true tentacle in *Halistemma*. What Claus describes as *Halistemma tergestinum*† does not seem to belong to *Halistemma* in the signification given to the generic name by its founder, Huxley. It belongs rather to Huxley's genus *Stephanomia* in all its structure, but especially in the character of its tentacular knobs, a feature of greatest importance in the classification of the Physophoridæ.

Haeckel (*Entwickelungsgeschichte der Siphonophoren*) proposes a division of the Agalmidæ which has some advantages, although to use the trifid character alone of the tentacular knob as a basis of his subfamily Crystallodacea separates those with an involucrum, and places *Agalmopsis* (*Stephanomia*, Huxley) with *Forskalia* and *Halistemma*. These last have no involucrum in the tentacular knob, and the former has sexual organs arising at the base of a polyp, while the latter has these same structures midway between two tasters. There does not

* Leuckart, Zur Nähern Kenntniss der Siphonophoren von Nizza, Taf. XII. fig. 15. When I studied Halistemma, I did not know of this difference of observation by Kölliker and Leuckart.

† 1. Metschnikoff, Proc. So. Fr. Nat. Moscow, Vol. VIII.; Studien der Medusen und Siphonophoren, Zeitsch. f. Wiss. Zool., Bd. XXIV.

2. Claus, C., Ueber Halistemma Tergestinum, &c., Wien, 1878. Mittheilungen über Siphonophoren und Medusen Fauna Triests, Zool. bot. Gesell. Wien, Tom. XXVI.

3. Eschscholtz characterized the genus Agalma, "Tentacula ramulis clavatis : clava apice bicuspidata."

seem to be sufficient ground for such a subdivision. I think it would be better if all were placed with *Athorybia*, as separate genera, in the Agalmidæ, and no subdivision of the group of any other kind at present attempted.

In the *Neue Beiträge*, Gegenbaur substitutes the name *Stephanomia* for that of *Forskalia* to designate a well-known form. He says, however, nothing about the genera *Halistemma* and *Agalmopsis*, and neither appears in his scheme of classification at the end of that work. Possibly he considers both as simply species of Agalma.

That which Claus in the last year (1879) has described and figured under the name of *Agalmopsis utricularia*, ought to be a new genus rather than a species of *Agalma* or *Agalmopsis*.*

There seems no reason why the name Stephanomia, which Lesson applied to both *Stephanomia contorta* and *Apolemia uvaria* of later authors, should designate the form with a biserial row of nectocalyces that it now does. Apolemia (Pl. I. fig. 1) is a well-marked genus. Leuckart adopts the name Forskalia of Kölliker in his *Siphonophoren von Nizza*, and in his *Zoologische Untersuchungen* applies the name Stephanomia to the same genus. He rightly says of the so-called *Forskalia* that it was first described by Milne-Edwards under the name of *Stephanomia contorta* (*Siphonophoren von Nizza*, p. 93).

St. delle Chiaje's use of the generic name, in a description of *Stephanomia ophiura*, although hardly accurate enough to be quoted in this discussion, should be mentioned. His designation of a species as *ophiura* is still retained in the nomenclature, and the form is easily to be known from *contorta*, from which even the fishermen of Messina distinguish it, although they affix to both a characteristic name, "Pinie di Mare."

Kölliker (*Siphonophoren von Messina*, p. 18) says that Lesson is wholly in error, "Wenn er die *Stephanomia contorta* und *prolifera* von Milne-Edwards zu derselben (*Apolemia uvaria*) zieht." In the *Nachtrag* to the same work he says: "Immer hin bleibe ich bei dem Genus Forskalia das nach einem vollständigen Thiere gebildet ist und kann der Name Stephanomia für das nur unvollständig bekannte Thier bleiben für das er von Peron zuerst aufgestellt wurde." The "unvollständig bekannte Thier" was that same form whose anatomy Huxley later published under the name which Peron gave it, although he says that Peron's sketch has "no scientific value." What animal Peron had will

* Claus, Agalmopsis utricularia eine neue Siphonophore des Mittelmeeres, Arbeiten aus dem Zoologischen Instituts der U. Wien und der Zoologischen Station in Triest, Bd. II. 2 Heft.

always remain problematical, and there is no good reason to identify the form studied by Huxley with it.

In the *Grundzüge der Zoologie*, 3 Auf., p. 237, Claus includes in the family of Agalmidæ *Forskalia* (*Stephanomia*, M. E.), *Halistemma*, and *Agalmopsis*. He, like Haeckel, mentions *Nanomia cara* as a species of *Halistemma*, and says that *Stephanomia* (Peron) is included in the same genus. Packard follows Claus in this reference of *Nanomia* to *Halistemma*.

In *Nanomia cara*, the first formed structure in the larva, according to Mr. Alex. Agassiz, is the float, as in *Agalmopsis* (*Stephanomia*, Metsch.). In Halistemma, according to Metschnikoff, the swimming-bell and float develop together from the very first. Although it is possible that the float is simply a modified Medusa bell or nectocalyx, no one would mistake the young of *Halistemma* for that of a *Nanomia* larva. As Metschnikoff has already pointed out, *Nanomia* in its younger stages resembles the genus *Agalmopsis* * (*Stephanomia*, Metsch.). Huxley's classification of the Siphonophoræ, with a verbal change, is the best which has been proposed as far as the Agalmidæ are concerned. We can retain the three generic names *Agalma*, *Agalmopsis*, and *Halistemma*. That would keep Eschscholtz's genus to designate a Physophorid with a trifid tentacular knob, the *Agalmopsis* of Sars with a single terminal filament on the same structure, and *Halistemma*, a form the tentacular knobs of which do not have involucra, and the tentacle is replaced by the pedicels of the tentacular knobs. In addition to the genera *Agalma*, *Agalmopsis*, and *Halistemma*, I would include *Athorybia* among the Agalmidæ, on account of its embryonic likeness to *Agalma*. It may possibly be simply the young of this genus. The only other Physophorid, except *Stephanomia* (*Forskalia*), where we have a multiserial necto-stem, is *Physophora tetra-*

* Notwithstanding Sars figures three radically different kinds of knobs in his genus *Agalmopsis*, a condition only observed, with this exception, in *Rhizophysa* and the larval forms of certain Agalmidæ, his figures 5, 6, on Plate V. are among the earliest, if not the first, representations of a tentacular knob with an involucrum and a single terminal filament. I retain, therefore, the name which he has given for the Jelly-fish with this characteristic, particularly on account of the exact use of *Stephanomia* by Milne-Edwards (Ann. d. Sci. Nat. 1841, Tom. XVI. p. 217). See also Leuckart's note, Siphonophoren von Nizza, p. 93 ; and Huxley, Oceanic Hydrozoa ; Sars, Fauna Littoralis Norvegiæ, 1846. In Middelhavet's Littoral Fauna, where all descriptions of Siphonophores are simply numbered, and with no subdivision, *Agalma rubrum* (*A. punctatum*, Köll.) is followed directly by *Agalma Sarsii*, a species with a trifid tentacular knob. In that work Sars makes no mention of the genus with a covered (by an involucrum) tentacular knob and a single terminal filament.

stica of Philippi (Müller's Arch., 1843). Leuckart thought (*Siphonophoren von Nizza*, p. 106, note 2) that this species ought to be made a new genus. I have not found the form redescribed by any naturalist since Philippi, and, although I have frequently taken *Physophora hydrostatica* and *Philippi* in my excursions on the Mediterranean, I have never seen *tetrastica*. Gegenbaur's view, to which Keferstein and Ehlers also incline (*Zoologische Beiträge*, p. 30, note 7) seems a good explanation of the apparently multiserial arrangement of nectocalyces spoken of by Philippi. Gegenbaur suggests that this multiserial character of the necto-stem in *tetrastica* is brought about by an accidental twisting of the necto-stem, a thing which often happens in *Physophora*, *Agalma*, and *Halistemma*. An *Agalma* which answers to Leuckart's description of *A. clavatum* was found in such numbers as to give me almost a perfect series between it and *Agalma Sarsii*. It was not possible, however, for me to raise the latter from the former, but the evidence which have mentioned seems enough to prove the identity of the two. Claus * has already made a similar suggestion. I have frequently taken at Villefranche a Jelly-fish identical, I think, with that which has been described by Claus as *Halistemma tergestinum*, and by Metschnikoff as *Stephanomia pictum*. A description of this animal, which I had formerly thought new to science, I had prepared without any intimation of the previous work of these naturalists. *S. pictum* was taken by Metschnikoff from the same locality where my studies were made. I think from the character of the tentacular knobs that we have in this interesting Siphonophore a true *Agalmopsis* as I have limited the genus, or a Physophorid with an elongated stem, no part of which is enlarged into a sac as in *Physophora*, and which is furnished with only a biserial row of nectocalyces. In addition it has a tentacular knob possessing an involucrum and a single filament. Metschnikoff's change of the Jelly-fish described by him, which is probably the same, from the genus *Halistemma*, to which he at first referred it, to the genus commonly known as *Stephanomia*, was well made.

The feature which distinguishes *Agalmopsis* (*Stephanomia*, Metsch.) *picta* from *Halistemma*, together with those already mentioned, is the position of the sexual organs (Pl. I. figs. 1, 3, 6), and, less definitely, the small size of the covering scales as compared with the nectocalyces. The crimson and orange sexual organs in *H. tergestinum*, as Claus figures them, and as I have also observed, are clustered, both male and female, at the base of a taster (Pl. I. fig. 6), the male mounted on an

* Zeitsch. f. Wiss. Zool., Bd. XII. p. 559.

especial stalk, and not separated from the taster, as in *H. rubrum.* The bracts are small, and so transparent that at first sight one is inclined to doubt their existence in *Agalmopsis picta*, while in *Halistemma* they are large and conspicuous. This feature effects very considerably the relative forms of the two Jelly-fishes.

All along the necto-stem and polyp-stem of *Agalmopsis picta*, more especially, however, upon the former, there are to be found in the ectoderm, as Claus has already mentioned, bright crimson pigment spots more clearly marked than is generally the case with similar spots on the stem of other Siphonophores. Two of these pigment spots, together with a finger-like process near them, also exist on the young nectocalyces. In very young swimming-bells there are three of these pigment spots. They occupy a position similar to that of the pigment spots of other hydroid Medusæ, at the junction of the lateral and superior * tubes with the circumvelar vessel. There are very interesting highly refractile red spots of a problematical function covering the bracts in *Agalma Sarsii* and *Agalma clavatum.* (Pl. I. fig. 2.) These bracts, from the place of attachment and the twisting of the stem, form a well-marked spiral around the polyp stem of the animal. The spots on each side of a central line are arranged on every scale in irregular rows, extending longitudinally across the bract, each pigment spot being enclosed in a cell. These peculiar pigment spots of the covering scales, represented remotely also in some genera, as in *Apolemia* (Pl. I. fig. 1), by elevations composed of clusters of cells on the surface of the bract, are the most apparent structures in the transparent bract of *A. Sarsii*, since with that exception there is hardly any coloration in the covering scale. In *A. clavatum*, the sexually mature young of *A. Sarsii*, only four rows of these pigment spots occur, as Leuckart has shown. When the bracts which bear these paralleled rows of spots are detached from the axis, their color changes to a yellow, and a fluid of the same color exudes into the surrounding water. I have not been able to find any mention of this rupture of the cell wall and discharge of a yellow fluid when the bract is detached, in the descriptions by other naturalists. I think these scale cells belong to the ectodermic layer.

* A nomenclature of the different spheromeres of the nectocalyx of a Siphonophore would simplify a description of the bell. As paired chymiferous tubes opposite each other have resemblances in their course from their relation to a plane passing through the dorsal and ventral line of the stem, they may be called lateral tubes, and the respective sections of the bell in which they lie, lateral spheromeres. The remaining spheromeres, according to their position in relation to a float, where such exists, may be called the superior, or the inferior, corresponding with a proximal and a distal.

The pigment spots mentioned in the nectocalyx of *Agalmopsis picta* have no resemblance to these peculiar bodies on the bracts, nor do they change their color when the swimming-bell is detached. The presence of such spots on the younger bell of *Agalmopsis picta*, and so little developed on the adult, rank them among patterns of embryonic coloration, examples of which are not unknown on other structures of these animals. *Stephanomia* * (*Forskalia*) has a similar large yellow spot, which persists in the adult nectocalyx, at the junction of radial and circular tubes.

The different stages in development of the female sexual organs of *Halistemma* have never been described or figured. Kölliker,† in his plate illustrating this genus, does not even represent these parts, and Leuckart‡ figures the female organs as a botryoidal structure, at the apex of a single polyp-like stalk. In several specimens, in addition to a structure of this kind, we have, as I have figured (Plate II. Fig. 3), others with the stalk on which the botryoidal mass is borne bifid at its extremity. This is probably simply another stage in development of these organs. As Leuckart well says, the sexual organs in *Halistemma* have no direct connection with the tasters; still, the female structures, at times, arise very near them.§

* The single yellow pigment spot at the junction of radial and circular tubes in *Stephanomia* (*Forskalia*) has on each side a finger-like process, and also, separated from these only by a short distance, an additional pair of the same rudimentary tentacles, as they may be called. The pigment spot is mentioned by Kölliker, who also calls attention to one pair of these tentacles or processes. He says : "Der Pigmentfleck ist insofern interessant als bei *keinerandern* Siphonophore Pigmentirungen de Schwimmglocken beobactet wurden." (Schwimmpolypen von Messina, p. 4.)

† Kölliker, Schwimmpolypen von Messina, Tab. IV.

‡ Leuckart, Zoologische Untersuchungen, Tab. II. fig. 14 ; Siphonophoren von Nizza, Tab. XII. fig. 15.

§ Claus says (*H. Tergestinum*, &c., p. 45) : "Wo man bei verwandten Agalmiden die Sprossung der Geschlechts-träubchen am Stamme beschreiben findet representirt entweder der Stiel des Träubchens einen Taster dessen Endabschnitt kurz und verkümmert bleibt oder aber der Tasterschlauch hat sich von Stiele gelöst und ist abgefallen." The resemblance to a taster of the stalk upon which the botryoidal female organs of *Halistemma* are borne, is very small. However, in *Agalmopsis* and *Stephanomia* (*Forskalia*) we find the sexual system at the base of the true taster, which seems to support Claus's suggestion. Huxley, who had not seen the genus *Halistemma* when "Oceanic Hydrozoa" was written, says of reproductive organs that they are like those of *Stephanomia*, and are attached directly to the cœnosarc. The sexual organs have no similarity in point of attachment, as can be seen from my figures of these two genera (Pl. II. figs. 1, 3) ; for while in the case of *Halistemma* they arise directly from the stem, in *Agalmopsis* (*Stephanomia*) they are united to the base of the taster.

V. Notice of a few Siphonophoræ and Velellidæ from the Eastern Coast of the United States.

Up to the present time few forms of either of these groups of Jelly-fishes have been described from the waters of our bays and sounds. They seem to be only occasional visitors, blown into the neighborhood of our shores from mid-ocean, or brought there from the tropics by the Gulf Stream. The wealth of tubular Medusæ which one finds in the Mediterranean is unknown on New England coasts or in Charleston Harbor, localities in which these animals have been best studied. Upon many single excursions on the quiet bays near Nice, in Southern France, I have taken eight different genera of Siphonophoræ; but their rarity is so great at Newport that seldom have more than one or two genera been taken by me in the same day; and a whole summer, in which I was almost daily upon the water, has passed without the observation of a single genus. A similar case of absence of all pelagic animals happened at Villefranche, last November. In that month, although I was on the water daily, I observed not only no Siphonophores, but also none of those Heteropods and Pteropods which later appeared in such numbers. Certain of the Siphonophoræ, however, are more abundant with us than in Villefranche, Naples, or Messina. *Physalia caravella* is now rarely taken in numbers by naturalists at either of these stations; but many examples of *Physalia arethusa* may be found almost any summer in Vineyard Sound or the entrances to Narragansett Bay.

The well-known *Physalia arethusa* is the most common of New England Siphonophores. It was long ago described by one of the pioneers in the study of Jelly-fishes, and later beautifully figured by Prof. Agassiz in the Contributions to the Natural History of the United States. Prof. McCrady* describes a form, *Physalia aurigera*, which is considered by Mr. Alex. Agassiz† as the same species. In the Catalogue of the North American Acalephæ, the list of places from which specimens of *Physalia arethusa* had been taken includes localities all the way from Cape Cod to Florida, and beyond in the West Indies.

The two floating Hydroids, *Velella* and *Porpita*, so closely allied to the Tubularians and known as the Velellidæ, are also found in our waters. The problematical genus *Rataria*,‡ by some supposed to be the young of *Velella*, in swarms of which it is generally found, and by others an immature *Porpita*, I think has not been described from our coast. I

* Gymnopthalmata of Charleston Harbor, 1857.

† North American Acalephæ, 1865.

‡ Pagenstecher, Zeitsch. f. Wiss. Zool., Bd. XII., 1863.

find no mention of it from New England waters. According to Agassiz, our *Velella* is *Velella mutica* of Bosc. Of that identification there seems no doubt, considering where the animal which Bosc described was found; but, as Pagenstecher * and Delle Chiaje suggest, it is difficult to see exactly what Bosc meant by his other species, *tentaculata*. The former of these authors says Bosc called the *Velella* of Linné and Lamarck *mutica*, while the species *spirans* of Forskal received the name *tentaculata*. Mr. Alex. Agassiz mentions a *V. septentrionalis* from our Pacific coast. Some of the material for the earliest descriptions of the Siphonophoræ and Velellidæ was collected in the Pacific Ocean, and near our western shores, and we should naturally expect these species taken by early voyagers from those localities.

Porpita I have never seen alive in our waters, but have a dried specimen preserved on paper after the manner of a plant, taken by a sailor not far from Nantucket. Prof. McCrady describes a species of *Porpita* from Charleston Harbor, not very different from Guilding's *Porpita* (*Polybrachionia Linneana*), which he calls *Porpita Linneana*. He is inclined to think it a new species.

The only known member of the long-stemmed Siphonophoræ, provided at one end with a float or air-bladder, which has been described from New England waters, is *Agalmopsis cara* (*Nanomia cara*, A. Ag.; *Stephanomia cara*, Metsch.; *Halistemma carum*, Haeckel, Claus, Packard, and others). This animal was first described by Mr. Alex. Agassiz, to whom we owe so much of our knowledge of the Jelly-fishes of our waters. The drawings and descriptions of the development which he gives are not only the earliest of this particular genus, but, with those of Claus, Leuckart, Kölliker, and Gegenbaur, of the embryology of the Siphonophoræ as a whole.

As I have already said, Haeckel considers *Nanomia cara* a species of *Halistemma*, and places it under this genus in his table of the Agalmidæ. He seems to have been followed by Claus, who adopts the name *H. carum* in his *Grundzüge der Zoologie*. When Mr. Agassiz described the form he said it was closely related to *Agalmopsis* as well as *Halistemma*, but that the mode of arrangement of the swimming-bells and the nature of the tentacles of the feeding polyps show undoubtedly that it cannot be placed in the same genus as *Agalmopsis*, having in mind Sars's genus. *Nanomia cara*, according to Metschnikoff, as already shown, should be regarded as a species in the genus *Stephanomia*. The reason for his conclusion, he says, is on account of the resemblance between the larvæ as figured

* Pagenstecher, Zeitsch. f. Wiss. Zool., Bd. XII., 1863.

by Mr. Alex. Agassiz and Kowalevsky. He says: "Die Aelteste von Kowalevsky gezogene Larve mit Luft apparat Magen und Fang faden gleicht so sehr dem jungsten von Alex. Agassiz gefangenen Jugendzustande der Nanomia, dass es mir sehr wahrscheinlich ist, dass auch diese Physophoride in die Gattung Stephanomia eingezogen werden muss zumal zwischen beiden eine grosse anatomische Analogie besteht." The absence of the cap-shaped provisional bell in the very young *Nanomia* shows that it does not belong to the genus *Agalma*, and the fact that a float and not a nectocalyx is first developed, separates it from *Halistemma*. Metschnikoff's conclusion seems to me the most natural one. I therefore would refer it to the genus *Agalmopsis*, of which I regard *Stephanomia*, as ordinarily used, a synonym.

There are certain points in which, following the description by Mr. Alex. Agassiz (North American Acalephæ, pp. 200 – 213), *Nanomia* differs from the other related Siphonophoræ which I have studied. He says that the float in this genus contained a globule of oil. I have never seen the genus fully grown in our waters, and can only judge from my studies of most of the other genera of the justness of Metschnikoff's criticism (Studien der Medusen und Siphonophoren, p. 36) of Alex. Agassiz on this point. If the float does contain oil, I think it an exceptional case among Siphonophores.

The second kind of feeding polyps, as described in *Nanomia*, are, I believe, simply immature forms of the first, and the tightly-coiled corkscrew parts are only undeveloped tentacular knobs. I have often found the young knobs of *Agalma Sarsii* and *A. elegans* clustered in the same manner at the base of a feeding polyp before a true tentacle had been formed.

The resemblance of the tentacular knob of *Nanomia*, with its "cnidofils," as shown in Mr. Agassiz's drawing (Fig. 339), to the provisional structures bearing the same name in *Agalmopsis picta* and the "Athorybia stage" of *Agalma*, is very great. This likeness is a very interesting fact, indicating either an embryonic condition of the adult of *Nanomia*, or that it is the larval form, sexually mature, of another Siphonophore.

The origin and earlier development of *Nanomia cara*, according to Mr. Agassiz, as a bud from the stem, is, I think, exceptional. In those other Siphonophores whose development is more or less completely known through the studies of Claus, Haeckel, Kowalevsky, and Metschnikoff, we find only an egg development of the new colony.

Dana describes (Mem. Amer. Acad., Vol. II. Part I.) a Physophorid from the Pacific Ocean. He calls it *Crystallomia polygonata*. The

figures which he gives of the tentacular knob seem to show that it is the genus *Agalma* of Eschscholtz. Haeckel refers it to his genus *Crystallodes.* The whole embryological history of *Agalma* and *Crystallodes*, with the exception of the appearance of a yolk-sac in the latter, according to Haeckel, as Metschnikoff says, is very much the same. Dana published his description in 1857, two or three years after the great works by the German naturalists on the Siphonophores of the Mediterranean.

I know of two genera of Leuckart's Calycophoridæ, a group of Siphonophores, which appears to me well defined, which have been described from our eastern coasts. In his "Gymnopthalmata of Charleston Harbor," Prof. McCrady describes and figures a new diphyozoid, which he names *Eudoxia alata*, and a new *Diphyes*, *D. pusilla.* His *Eudoxia alata* seems to be the same as *E. Lessonii* of Huxley. This animal, according to this prominent English naturalist, is the diphyozoid of *D. appendiculata*, a synonym of Leuckart's *D. acuminata.* The mention which Prof. McCrady makes of *Diphyes pusilla* is too short to be of service in distinguishing it from Mediterranean Diphyidæ. A figure of a *Diphyes acuminata* from Villefranche may have some interest, especially as its diphyozoid, *Eudoxia Lessonii*, has been found by me at Newport. Leuckart mentions in his *Siphonophoren von Nizza* an *Epibulia* (*Galeolaria*), given him by Philippi, and taken from the coast of Greenland.

To the Siphonophorous fauna of eastern coasts of North America * I can add a new member of the Agalmidæ, probably the same as Sars's *Agalmopsis elegans*, and the two diphyozoids, *Eudoxia Lessonii* and *Diplophysa inermis.* There is a great diversity of opinion among naturalists what Diplophysa is. All seem to be united in the opinion that it is a diphyozoid, but there is an unanswered question of what Calycophore it is the fragment. I mention a few of the opinions. Gegenbaur,† who first described the form, seems to think its resemblance not very distinct from *Ersæa truncata* of Will. On page 366 of his *Neue Beiträge* he says that "Sie (Diplophysæ) entsprechen in der Sculptur der Diplophysen-gattung Praya." *Praya* is probably the same as *Ersæa.* Huxley (Oceanic Hydrozoa, p. 66) says that *Diplophysa inermis* has some resemblance to the diphyozoid *Cucubalus* described by Quoy and Gaimard, but says he was unable to arrive at any definite opinion as to what animals were included by the French voyagers in their genera *Cymba* and *Cucubalus.*

* My observations on American Siphonophores were made in the laboratory of Mr. Agassiz, at Newport, R. I.

† Beiträge zur näheren Kenntniss der Schwimmpolypen (Siphonophoren).

The title of one of Claus's valuable papers on the Siphonophoræ is *Die Gattung Monophyes und ihr Abkömmling Diplophysa*, in which he supports the idea that Diplophysa is a diphyozoid of *Monophyes gracilis*, Cls. He makes Huxley's genus *Spheronectes* a synonym of *Monophyes*. He repeats in his *Grundzüge der Zoologie*, 3 Auf., 1876, that *Diplophysa inermis* is a diphyozoid of *Monophyes gracilis*, as stated above.

Metschnikoff (Studien über die Medusen und Siphonophoren, p. 46) says, concerning the relationship of these animals, that fragments of the form *Praya inermis* were described by Gegenbaur as *Diplophysa inermis*. He bases his idea of the relationship of these two genera on the identity of the larva of *Praya*, which he describes, with the remarkable genus of Gegenbaur, and more especially on the resemblance in the form of their nectocalyces. He adds also, that both genera are of small size, which cannot, if taken alone, be a very strong argument for their relationship.

TABULAR LIST OF VELELLIDÆ AND SIPHONOPHORÆ,

FROM THE EASTERN COAST OF THE UNITED STATES.

VELELLIDÆ.

Velella mutica, Bosc.
 Agassiz, L., Cont. Nat. Hist. U. S., Vol. IV. p. 366, 1862.
 Agassiz, A., North American Acalephæ, p. 216, 1865.
Velella spirans.
V. tentaculata (?), Bosc.
Porpita Linnæana, Less.
 McCrady, Gymnopthalmata of Charleston Harbor, 1857.
 Agassiz, A., North American Acalephæ, 1865.
Porpita gigantea.
 Fewkes, Nantucket.

SIPHONOPHORÆ.

I. Physophoridæ.

1. Agalmidæ.

Agalma elegans, Fewkes, Newport.
Agalmopsis elegans, Sars, Fauna Littoralis Norvegiæ, 1846.
Agalmopsis (sp. ?).
Nanomia cara, Agassiz, A., North American Acalephæ, 1865.
Halistemma carum, Haeckel, Ent. d. Siphonophoren.
Stephanomia cara, Metschnikoff, Zeitsch. f. Wiss. Zool., Bd. XXIV., 1874.

2. Physalidæ.

Physalia arethusa, Til.
 Agassiz, L., Cont. Nat. Hist. U. S., 1862.
 Agassiz, A., North American Acalephæ, 1865.
Physalia aurigera, McCrady.
 Mr. Agassiz suggests that this is the same as *Physalia arethusa* of Tilesius.

II. Calycophoridæ.

1. Diphyidæ.

Diphyes acuminata, Leuck. The diphyozoid of this Siphonophore is *Eudoxia campanulata.*

Eudoxia campanulata, FEWKES, Newport.
Eudoxia Lessonii, HUXLEY, Oceanic Hydrozoa.
Eudoxia alata, MCCRADY, Gymn. of Charleston Harbor.

Diphyes pusilla, MCCRADY, Gymn. of Charleston Harbor.

2. PRAYIDÆ.

Praya inermis has, according to METSCHNIKOFF, the diphyozoid *Diplophysa inermis* (GEG.).
Diplophysa inermis, FEWKES, Newport.

In this incomplete list of tubular Jelly-fishes, we miss many of those beautiful forms which are so familiar to the naturalist on the Mediterranean. Extended observations in our Southern bays will probably bring to light the well-known Siphonophores common to all oceans, *Apolemia*, *Abyla*, *Physophora*, and *Gleba*. Some of these have already been taken in the Gulf of Mexico and Caribbean Sea. *Rhizophysa*, found in the same localities, may also be expected, brought by ocean currents to our coasts.

CAMBRIDGE, April 1, 1880.

EXPLANATION OF THE PLATES.

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a, float; *b*, nectocalyx; *c*, necto-stem; *d*, polyp-stem; *e*, feeding polyp; *f*, taster; *g*, ovaries; *h*, testes; *i*, tentacle; *j*, tentacular knob; α, involucrum; β, sacculus; γ, pedicel; δ, terminal filaments; *k*, tentacle of the taster; *l*, somatocyst; *n*, radial tubes; *o*, circular vessel; *p*, covering scale; *q*, longitudinal canal; *ec*, ectoderm; *en*, entoderm; *r*, joint in polyp-stem; *s*, nectocalyx of diphyozoid; *t*, crescentic-formed velum in Gleba.

The mantle-tubes, somatocyst, and central tube of the bract or covering scale are designated by the letter *l*. They seem to be the same structures.

PLATE I.

Fig. 1, *Apolemia uvaria*, a part of the polyp-stem, magnified four diameters. The longest stem observed by me was eight feet in length. Figs. 2, 3, 4, 5, 6, 7, different stages of development of the tentacular knob of *Physophora hydrostatica*. Fig. 3 shows the origin of the involucrum. Fig. 4, 5, 6, represents the provisional form of the knob, and the embryonic terminal filaments. Fig. 7, a knob in the most developed condition.

PLATE II.

Fig. 1, view of *Agalmopsis picta* from one side, magnified two diameters. The tentacles are drawn to the vicinity of the polyp-stem by which the tentacular knobs appear on the upper side of that appendage (an unusual condition.) Fig. 2, covering scale of *Agalma Sarsii*. Fig. 3, portion of the polyp-stem of *Halistemma rubrum*, magnified four diameters. Fig. 4, tentacular knob of *Halistemma rubrum*. The lower extremity of this figure joins figure 3 at the point γ. Fig. 6, taster of *Agalmopsis picta*. This figure shows the position of the male and female organs in reference to the taster.

PLATE III.

Fig. 1, *Praya diphyes*. Fig. 2, *Praya*, sp. (?) This unknown species of Praya differs from *Praya cymbiformis* in the equality in size of the nectocalyces, their triangular outline when seen in profile, and the direct course from junction to circular vessel of the radial tubes. The difference between it and *Praya diphyes* is plainly brought out by the accompanying Fig. 1. It has the somatocyst in but one nectocalyx, and the diphyozoids are crowded together along the polyp-stem, somewhat similar to the conditions among the Agalmidæ. I incline to regard Fig. 2 as the young of *Praya cymbiformis*. Fig. 3, *Diphyes acuminata*. Fig. 4, lateral view of the nectocalyx of *Gleba hippopus*. Fig. 5, inferior view of a similar nectocalyx. Fig. 6, *Spheronectes* (*Monophyes*) *inermis*. All these drawings are from Jelly-fishes taken in the Mediterranean.

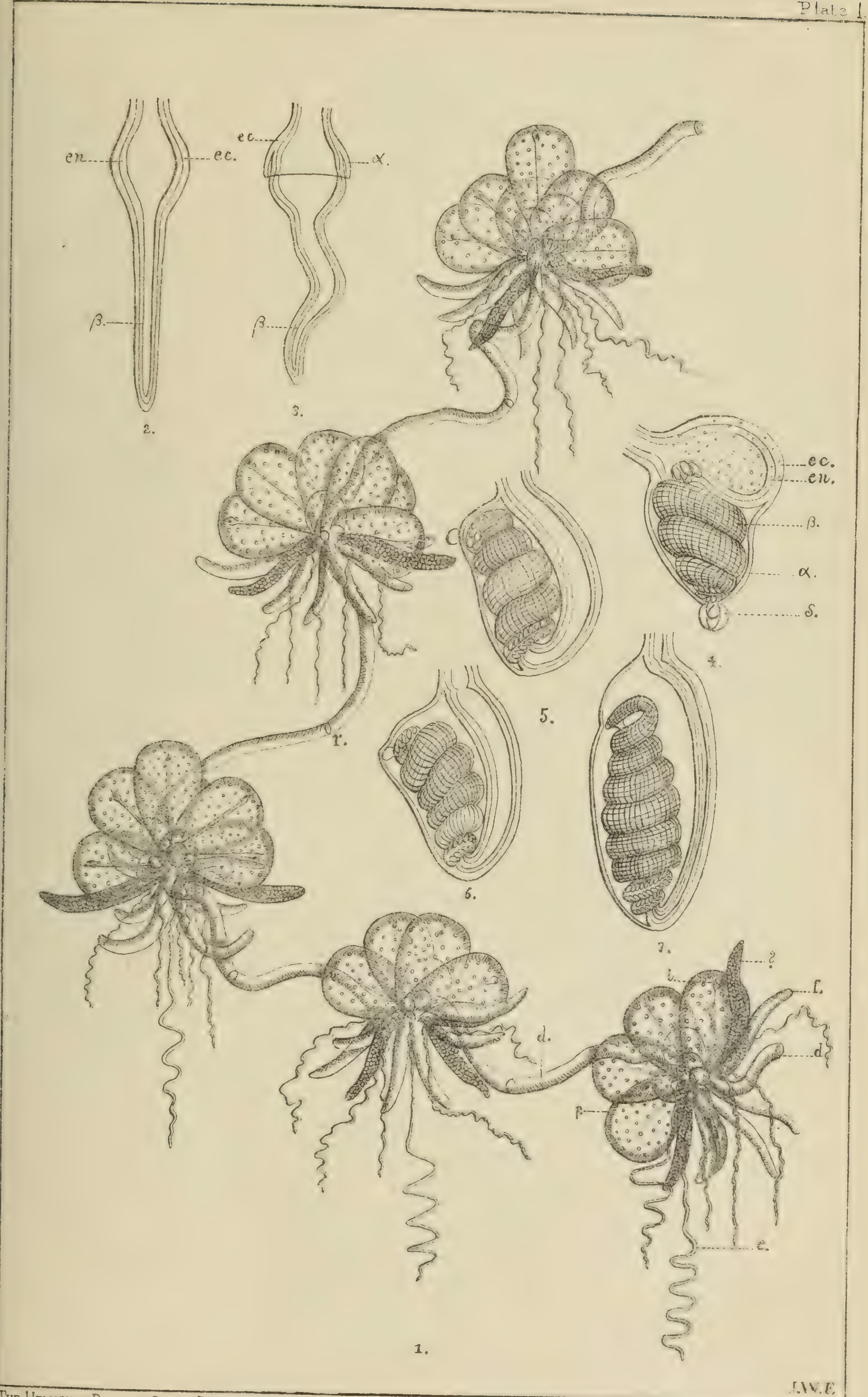

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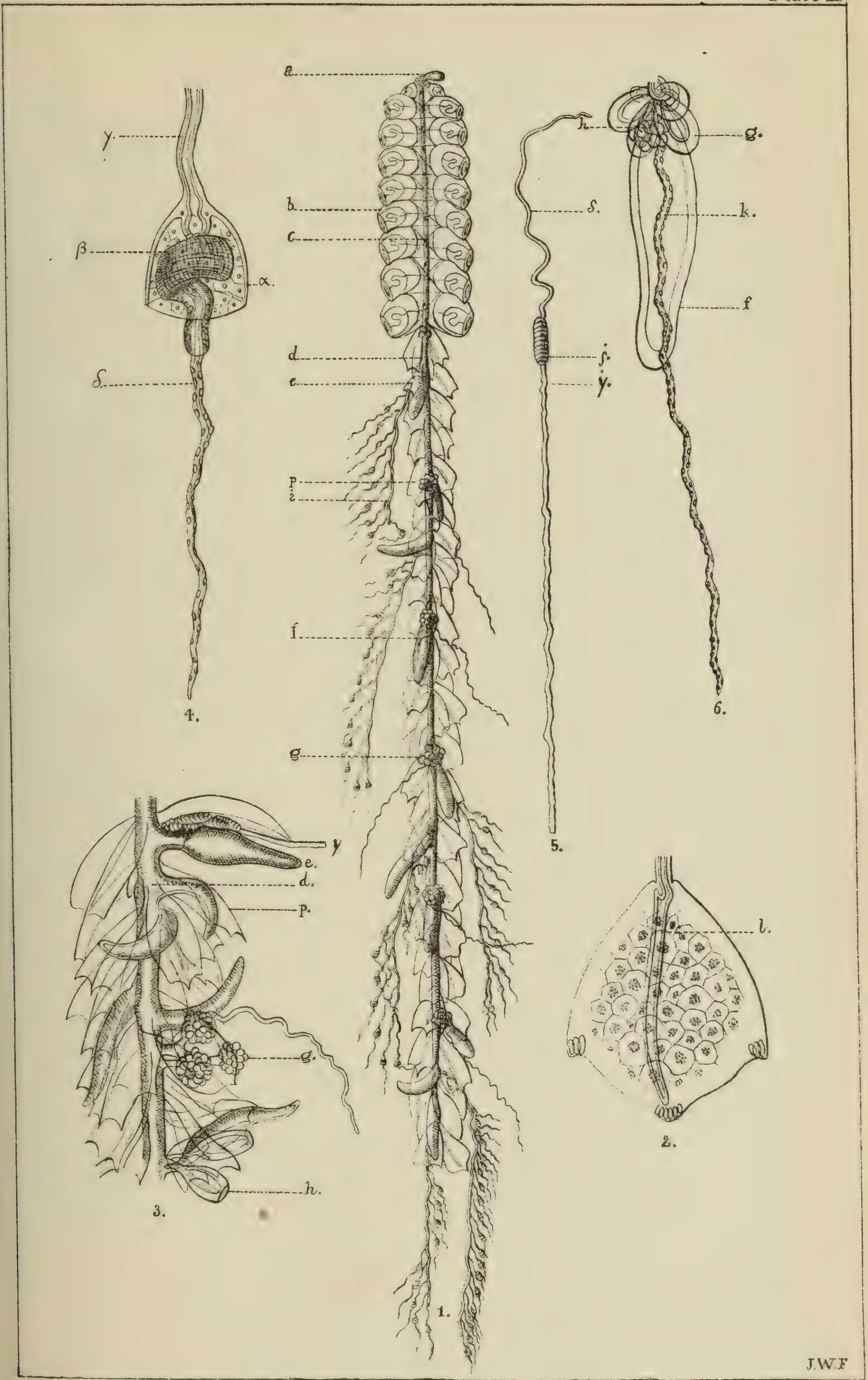
Plate II.



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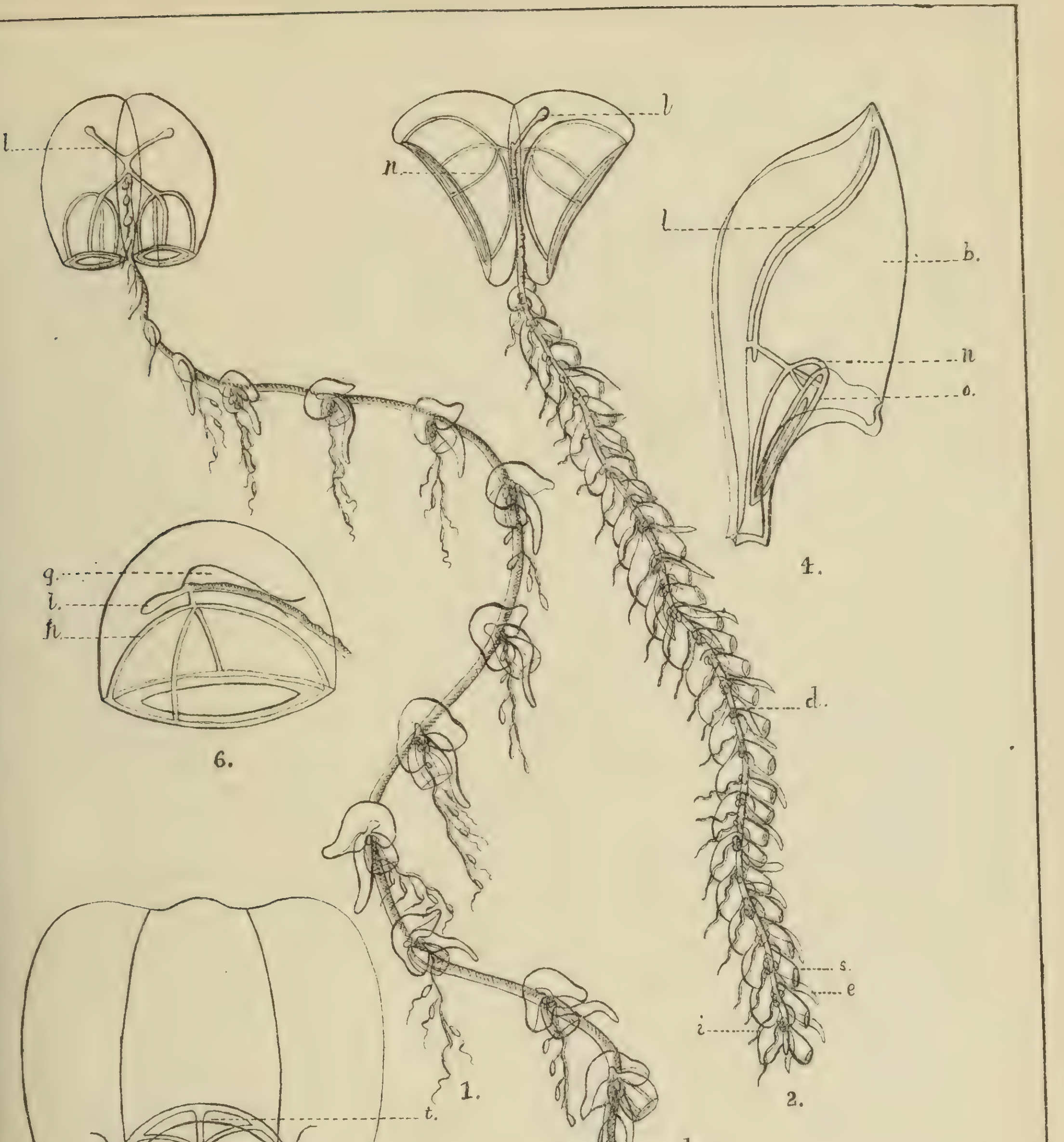

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No. 8. — (Letter No. 4.) *To* Carlile P. Patterson, *Superintendent United States Coast and Geodetic Survey, Washington, D. C., from* Alexander Agassiz, *on the Dredging Operations carried on during part of June and July*, 1880, *by the United States Coast Survey Steamer "Blake," Commander* J. R. Bartlett, *U. S. N.*

I joined the "Blake," at Newport, late in June. According to your instructions, we proceeded to the northeastern edge of George's Shoal, where we ran our first line of dredgings from the 100-fathom line to a depth of nearly 1250 fathoms. Our second line was run to the southeast, off Montauk Point. This was interrupted by bad weather. We were compelled to put into Newport, and completed the line on our return from the South. This line extended to nearly 1400 fathoms.

On leaving Newport for the second time we steamed directly for Charleston, S. C. A line of dredgings was run from the 100-fathom line normal to the coast directly across the Gulf Stream to a distance of about 120 miles to the eastward of Charleston. Finding that our depth did not increase at that distance, — our greatest depth not being much more than 350 fathoms, — Commander Bartlett thought it prudent to return towards shore, to the so-called axis of the Gulf Stream, and to run a line in a northeastern direction parallel to the coast in the trough of the Gulf Stream. To our great astonishment the depth did not increase. We carried from 250 to less than 300 fathoms until we nearly reached the latitude of Cape Hatteras, when in a short distance there was a very rapid drop from 352 fathoms to 1,386 fathoms. A fifth line was run normal to this northern slope of the Gulf Stream plateau, to a depth of 1,632 fathoms. A sixth line was run to the northward of Cape Hatteras, to a depth of 1,047 fathoms. A seventh line was run east off Cape May, from the 100-fathom line to nearly 1200 fathoms.

We were greatly disappointed in the richness of the fauna on the lines off Charleston and in the Gulf Stream, owing partly to the very gradual slope of the continent towards deep water, and the strong current of the Gulf Stream, which sweeps everything off the bottom along its course. There is but little food for the deep-water animals, and it was only along the edges of the Gulf Stream where mud and silt accu-

mulated that we made satisfactory hauls on our Southern lines. What was obtained seemed to be a scanty northern extension of the fauna of the Caribbean Sea and of the Gulf of Mexico between the 100 and 350 fathom lines. It was not until we trawled on the steep slope of the Gulf Stream plateau south of Cape Hatteras, where the bottom was fine mud and Globigerina ooze, that we made a rich harvest again, in striking contrast to the poor hauls along the well-swept rocky or hard bottom of the Gulf Stream to the southward. Along the western edge of the Gulf Stream we came upon several patches of the modern green-sand formation, where the bottom was entirely composed of perfectly clean dead Globigerinæ. Although Pteropods were very common at the surface all the way from Charleston to Cape Hatteras, they were only rarely brought up dead from the bottom; but when the steep slope south of Hatteras was reached they again assumed a prominent part in the composition of the bottom mud.

While running the line parallel to the coast from off Charleston to Cape Hatteras, we came twice upon localities where the sounding cup brought up nothing but clean Globigerinæ, the bottom consisting entirely of the modern green-sand to which Bailey and Pourtalès had already called attention as forming off shore on the Atlantic coast of the United States. The rapid changes in the character of the mud, as we increase both our distance from shore and the depth, are well shown in the nature of the bottom of the different depths along the short, steep line forming the northern slope of the Gulf Stream plateau traced by the "Blake" from Charleston to south of Cape Hatteras. We very rapidly pass from the comparatively coarse mud to fine and finer ooze, which becomes an impalpable silt in the deeper water beyond 1,000 or 2,000 fathoms, assuming at the same time gradually a lighter color.

Among the Tunicates I may mention two new species of Salpæ, one of which is interesting, its chain occupying an intermediate position between that of Salpa pinnata and the ordinary Salpa chain of S. zonaria or S. Cabotti of our coast. The solitary individuals are gigantic specimens, measuring no less than twelve inches in length. This solitary form is closely allied to S. maxima, but differs from it in the number and arrangement of the muscular bands. The chains grow to a great length, some of them measuring more than ten feet in length and as much as nine inches in breadth. The zoöids are arranged as in S. pinnata, side by side in a single row, extending vertically across the whole width of the chain, and forming a thin ribbon, which when floating is usually slightly coiled like a tape. The zoöids of the chain resemble

S. Africana. This species was found at sea from Cape Hatteras as far north as the eastern extremity of George's Shoal.

Among the Acalephs, the most interesting form was a species of Dodecabostricha, Br., the largest specimen measuring no less than nine inches in height. Several specimens of a dark violet (claret-color) were brought up in the trawl, and it is very probable from the systematic affinities of this Medusa that, like its allies, the Rhizostomæ, it lives on the bottom, rarely coming to the surface. For the genus Dodecabostricha Professor Agassiz established a new family, the Brandtidæ, and placed it in the vicinity of the Charibdeidæ. While it undoubtedly has a general resemblance to the Charibdeidæ, the structure of the genital pouches and of the lobes of the actinostome shows that it is intermediate between the Aureliæ and the Rhizostomæ proper, combining at the same time structural features only found in the Pelagiæ. It is not known where Mertens found the species which is figured in Brandt's memoir. As we trawled mainly on mud or clay bottoms, but few Hydroids were collected.

All along the course of the stream we found large quantities of Trichodesmium erythræum. On one occasion, north of Hatteras, we passed through an extensive patch of this pelagic Alga, which colored the surface of the sea a dirty yellow for a distance of about a quarter of a mile by a hundred yards in width.

Among the corals a fine species of Flabellum, probably the Flabellum alabastrum, Mos., and a few species characteristic of the West India seas and of the Gulf of Mexico were found to extend as far north as Cape Hatteras. There were a number of Pennatulæ and Virgulariæ collected, probably the same species already described by Professor Verrill from the collections made by the United States Fish Commission, as well as a few Gorgoniæ, among which I may mention numerous specimens of Keratoisis. The Pennatulæ and Gorgoniæ were all remarkable for their brilliant bluish phosphorescence, a single Pennatula lighting up a large tub of water. A couple of species of Zoanthus were found in deep water. Among the Actiniæ large specimens of Bunodes and of Edwardsiæ came up from depths of from 600 to 800 fathoms.

Among the Echinoderms all the way from Cape Hatteras to the extremity of George's Shoal, Ophiomusium Lymani were quite common in deep water. Echinus norvegicus is abundant, and Schizaster fragilis extends from deep water inside the 100-fathom line. A species of Asthenosoma and one of Phormosoma were also found in deep water, having the same general distribution as Ophiomusium. A fine species

of Urechinus closely allied to Urechinus naresianus, and several of the rarer species of Starfishes, — Archaster, Porcellanaster, Luidia, Astrogonium, Porania, Pteraster, and Hippasteria, — were found to extend far into deep water; and beyond 1,000 fathoms, off George's Bank, we found several fine specimens of Brisinga, as well as three or four species of the remarkable deep-sea Holothurians belonging to the order of Elasmopoda; among the Crinoids, Comatula Sarsii? and a few specimens of Rhizocrinus. Although the line to the eastward of Charleston, S. C., was commenced off the very home of the Scutellæ and other Clypeastroids, it is remarkable that not a single Mellita or Clypeaster was dredged up, either on that line or the line run in the axis of the Gulf Stream as far as Cape Hatteras. Echinarachnius off George's Shoal was found to extend to a much greater depth, living specimens having come up in the trawl from a depth of 524 fathoms.

But few Annelids were collected, a few specimens of Nemerteans, and of Calymne; one of the large Eunicidæ, the tubes of which, sometimes fully fifteen inches in length, often filled the bottom of the trawl when dragging on muddy bottoms, was specially numerous.

A number of species of Cephalopods, mainly Northern species already found in shallower waters by the United States Fish Commission, were brought up, many of them from considerable depths. The Gasteropods and Acephala were represented by many of the species collected by the "Lightning" and "Porcupine," and by the United States Fish Commission.

Among the Crustacea the most characteristic types were the gigantic Pygnogonidæ, a species of Willemoesia, a couple of species of Gnathophausiæ, Scalpellum, and large Amphipods.

Among the Fishes a large collection was made, mainly of Macrouridæ, including a few new genera, which will be described by Mr. Goode, of the United States Fish Commission. We found cod, extending to a depth of over 300 fathoms (off George's Shoal). Myxine and Lophius were brought up from 360 fathoms, as well as Sebastes norvegicus. A species of Phycis, from a depth of 233 fathoms, was found to be electric, giving quite a strong shock to Commander Bartlett and myself. It is a small species, about nine inches in length, of a light ashy violet color, with dull yellowish spots along the sides.

The absence of siliceous and other sponges in the collections made during this summer is very striking, and although the number of specimens of certain species was often very great, yet the continental faunæ of the northern part of the east coast of the United States is poor when

compared to the wealth of species found in the Caribbean Sea and Gulf of Mexico during the former cruises of the "Blake."

Commander Bartlett did everything in his power to make up for the absence of my assistant, and I was fortunate in again finding on board the older officers of the "Blake," Messrs. Sharrer, Jacoby, Peters, and Reynolds, whose industry, energy, and interest in the work has never flagged, and who have now attained a proficiency in deep-sea dredging hardly deemed possible three years ago. Lieut. Mentz and Dr. Persons joined the "Blake" during the winter of 1879, and Mr. Duvillard was attached to the "Blake" as recorder during the first part of our cruise. During this short cruise we made no less than fifty hauls: we accomplished nearly as much as during the three months of the first cruise in the Gulf of Mexico.

As the greater part of the collections made during this cruise of the "Blake" cover the extension into deep water of the ground already in part occupied by the United States Fish Commission, I have arranged with Professor Baird to send the bulk of the collections made north of Cape Hatteras, for final study, to some of the naturalists to whom the collections of the Fish Commission have been intrusted.

During the winter of 1879–80, Commander Bartlett, while sounding in the Western Caribbean Sea, made some twenty hauls with the trawl, dredge, and tangles. These collections, made incidentally by the officers of the "Blake," show the extension of the continental fauna of the Eastern Caribbean to its extreme western portion. Pentacrinus was found off Santiago de Cuba, and off Kingston, Jamaica. The deep-water fauna was found to be the same as the deep-water fauna of the Eastern Caribbean.

Mr. Bartlett showed that a strong current passing over a ridge, as in the case of the Windward Passage between Cuba and San Domingo, swept it entirely clean, so that but little animal life was found to live upon it. But immediately beyond this, on the Caribbean side, the mud and silt are deposited in great quantities and animal life becomes plenty again. This, as I have stated above, was also our experience during the present cruise of the "Blake," while dredging along the so-called axis of the Gulf Stream.

Lieut.-Commander C. D. Sigsbee accompanied us on the "Blake," to superintend in person the first trial of his collecting cylinder. It was sent down in 30 fathoms, from 5 to 25 fathoms, with quite a fresh breeze blowing, at about eleven in the morning, in full sunlight, — a time when, with a smooth sea, the pelagic animals would all have been found

on the surface. The cylinder was found to work most satisfactorily, and brought up a few Calani, Hydroid Medusæ, such as usually occur at the surface. A few slight modifications were suggested by Mr. Sigsbee, and Commander Bartlett recommended the addition of a wire-gauze trap, to facilitate the washing out of the microscopic animals which might be collected.

On the 1st of July the Sigsbee cylinder was tried for the second time in Lat. 39° 59′ 16″ N., Lon. 70° 18′ 30″ W., in 260 fathoms of water. The surface was carefully explored with the tow-net, to see what pelagic animals and others might be found on the surface. There were found Calanus, Sagitta, Annelid larvæ, Hydroid Medusæ, Squillæ embryos, Salpæ, and a few Radiolarians. The cylinder, filled with water which had been carefully sifted through fine muslin, was then attached to the dredging wire, and lowered, so as to collect the animals to be found between 5 and 50 fathoms. The time occupied by the cylinder in passing through that space was 28 seconds. The cylinder was then brought up, and the sieves and gauze trap carefully washed with water, which had also previously been strained through fine muslin. The water was carefully examined, and we found the very same things which had a short time before been collected at the surface with the tow-net and the scoop-net: nothing different was collected by the cylinder. The Radiolarians (two genera) were perhaps more numerous than at the surface. A slight breeze having sprung up after the surface collections had been examined, the cylinder was then sent down a second time at this same station, so adjusted as to collect any animal life to be found from a depth of 50 to 100 fathoms. Not only in this experiment, but in all the subsequent ones, the same precautions were taken in regard to straining the water which filled the cylinder at the start, as well as that used for washing out the sieve and the gauze trap. The messenger sent down to detach and open the machine occupied 21 seconds in reaching the (50 fathoms) point to which the cylinder was attached, and the cylinder then occupied 30 seconds in passing to the stop at 100 fathoms. On examining the sieves, it was found that the more common surface things, Calanus, Sagitta, Annelid larvæ, Hydroid Medusæ, and Squillæ embryos, were entirely wanting, and there were only two Radiolarians of the same species as those from the upper levels found after a careful scrutiny of the water. Nothing additional was brought up. The cylinder was then sent down a third time, lowered to a depth of 100 fathoms, the messenger sent down to open it (time occupied 45″), and the cylinder travelled from 100 to 150 fathoms (time 45″), so as to collect the animal life to be obtained

between these limits. On drawing up the cylinder and washing out the sieve of the trap, not only did we find that the water contained nothing different from what had been brought up by the cylinder from the lesser depth, but it did not contain even a single Radiolarian.

On the 15th of July, in Lat. 34° 28′ 25″ N., Lon. 75° 22′ 50″ W., we tried the Sigsbee cylinder for a third time, in a depth of 1,632 fathoms. With the same precautions before and after using it, the cylinder was sent to collect first between 5 and 50 fathoms (time 30″). The surface was somewhat ruffled, and but little was found on the surface beyond a few Crustacean larvæ and Heteropods. The cylinder contained Hydroids, fragments of Siphonophores, pelagic Algæ, Crustacean larvæ, and Heteropod eggs; forms which differed from these scooped at the surface, but were identical with the species found on previous days at the surface under more favorable surface conditions of the sea. Next, the cylinder was arranged to collect between 50 and 100 fathoms (time of messenger 21″ from surface to 50 fathoms, time of cylinder 40″ to stopper from 50 to 100 fathoms). The water was found to contain only a couple of Squilleæ larvæ, similar to those fished up at the surface. The third time the cylinder went down at this station it was lowered to collect from 100 to 150 fathoms (time of messenger from surface to 100 fathoms 45″, time of cylinder in passing from 100 to 150 fathoms 45″). The water when examined contained nothing. No Radiolarians were found at this station, either at the surface or at any depth to which the cylinder was sent (150 fathoms).

The above experiments appear to prove conclusively that the surface fauna of the sea is really limited to a comparatively narrow belt in depth, and that there is no intermediate belt, so to speak, of animal life, between those living on the bottom, or close to it, and the surface pelagic fauna.

The experiments of using the tow-net at great depths (of 500 and 1,000 fathoms), as was done by Mr. Murray on the "Challenger," were not conclusive, as I have already pointed out on a former occasion, while the so-called deep-sea Siphonophoræ, taken from the sounding line by Dr. Studer on the "Gazelle," may have come, as I have so often observed in the Caribbean, from any depth. I do not mean, of course, to deny that there are deep-sea Medusæ. The habit common to so many of our Acalephs (Tima, Æquorea, Ptychogena, etc.) of swimming near the bottom is well known; Dactylometra moves near the bottom, and Polyclonia remains during the day turned up with the disk downwards on the mud bottom. I only wish to call attention to the uncertain methods adopted for ascertaining at what depth they live.

As far as the pelagic fauna is concerned, those who have been in the habit of collecting surface animals know full well that the least ripple will send them below the reach of commotion; Müller and Baur were the first to adopt the use of a tow-net sunk below the surface to collect pelagic animals when the water was disturbed. It seems natural to presume, as we have found from our experiments with the Sigsbee cylinder, that this surface fauna only sinks out of reach of the disturbances of the top, and does not extend downward to any great depth. The dependence of all the pelagic forms upon food which is most abundant at the surface, or near it, would naturally keep them where they found it in greatest quantity.

Of course, with the death and decomposition of the pelagic forms, they sink to the bottom fast enough to form an important part of the food supply of the deep-sea animals, as can easily be ascertained by examining the intestines of the deep-water Echinoderms. The variety and abundance of the pelagic fauna, and its importance as food for marine animals, are as yet hardly realized.

One must have sailed through miles of Salpæ with the associated Crustacean, Annelid, and Mollusk larvæ, the Acalephs, especially the oceanic Siphonophores, the Pteropods and Heteropods, with the Radiolarians, Globigerinæ, and Algæ, to form some idea how rich a field still remains to be explored. The variety of the pelagic fauna in the course of the Gulf Stream is probably not surpassed by that of any other part of the ocean.

NEWPORT, R. I., August 20, 1880.

No. 9. — *Reports of the Results of Dredging, under the Supervision of* ALEXANDER AGASSIZ, *on the East Coast of the United States, by the U. S. Coast Survey Steamer "Blake," Commander* J. R. BARTLETT, *U. S. N.*

VII.

Description of a Gravitating Trap for obtaining Specimens of Animal Life from Intermedial Ocean-Depths. By LIEUT.-COMMANDER C. D. SIGSBEE, *U. S. N.*

THE old practice of dragging for animal forms at intermedial depths by means of a tow-net, which, during the several operations of lowering, dragging, and hauling back remained open, was not regarded by Professor Alexander Agassiz as affording acceptable evidence of the habitat of such specimens as were obtained, and he frequently referred to the subject during our association on board the "Blake" in 1878.

In March, 1880, it having been arranged that Professor Agassiz should make another cruise on board the "Blake," Commander J. R. Bartlett, U. S. N., commanding, he asked my co-operation in devising an apparatus to meet the rigid demands of the work in question. This resulted in the apparatus described herein, which is presented in the precise form used with success by the "Blake," although, as may readily be seen, it is open to great improvement, especially in minor details.

The "Challenger" had examined intermediate depths by means of tow-nets trailing from the dredge-rope while hauling the dredge or trawl. In such a practice it must have been that the depths to which the nets were sunk depended in some degree on the amount of slack rope payed out, and also on the strain upon the dredge-rope due to the resistance encountered by the dredge when dragging; it cannot therefore be said that strictly determinate depths were examined by that method, even assuming that the nets gathered nothing while being lowered and hauled back.

It occurred to me that by using an apparatus in connection with a line and lead, payed out vertically as in sounding, and by dragging vertically, instead of horizontally as formerly, there would be at least as much certainty with regard to depths as in the old method, and that simple mechanical devices could be invented to satisfy the conditions of

the work. The scheme has been stated in my volume on "Deep-Sea Sounding and Dredging," (p. 145, foot-note,) as follows: —

"Our plan is to trap the specimens by giving to a cylinder, covered with gauze at the upper end and having a flap valve at the lower end, a rapid vertical descent between any two depths, as may be desired; the valve during such descent to keep open, but to remain closed during the processes of lowering and hauling back with the rope. An idea of what it is intended to effect may be stated briefly thus: — Specimens are to be obtained between the intermediate depths *a* and *b*. The former being the uppermost. With the apparatus in position, there is at *a* the cylinder suspended from a friction *clamp* in such a way that the weight of the cylinder and its frame keeps the valve closed; at *b* there is a friction *buffer*. Everything being ready, a small weight or messenger is sent down, which on striking the clamp disengages the latter and also the cylinder, when messenger, clamp, and cylinder descend by their own weight to *b*, with the valve open during the passage. When the cylinder-frame strikes the buffer at *b*, the valve is thereupon closed, and it is kept closed thereafter by the weight of the messenger, clamp, and cylinder. The friction buffer, which is four inches long, may be regulated on board to give as many feet of cushioning as desired."

The following detailed description refers to the accompanying plate.

The copper cylinder A, riveted to the wrought-iron frame B, has a flap or clapper valve, C, opening inwards and fastened to the inner arms of the lever D D, the latter pivoting at E. The upper end of the cylinder is covered with the removable wire sieve F (60 wires to the inch), and inside the cylinder are the wire sieve G (27 wires to the inch) and the wire funnel or trap H (27 wires to the inch).

The steel wire rope on which the cylinder travels is placed in the loops I I, at the upper and lower extremities of the frame, and is retained therein by the screw-bolts J J.

The friction *clamp* is composed of the frame K, the two sliding chocks L and M, the adjusting screw N, the guide screws O O, and the eccentric tumbler P.

The friction *buffer* is composed of the frame Q, the two sliding chocks R and S, the adjusting screw T, the steel compression spring U, working in a chamber, and the regulating screw V. The bearing faces of the two sliding chocks are corrugated, and the inward movement of each chock is limited by a stud forming part of the frame and fitting loosely within a slot in the chock. In clamping the buffer to the rope, the chock R is always screwed in until stopped by its stud; the steel rope

is therefore always pressed between the two chocks by the elastic force of the spring, which may be regulated as desired. To regulate the buffer for any definite frictional resistance, clamp it to the rope, and move the regulating screw V well inwards; then suspend from the buffer a weight equal to the resistance decided upon. Move the regulating screw outwards until the buffer slides down the rope under the influence of the suspended weight. Since the chock R is always screwed "home" in clamping to the rope, the buffer remains regulated for prolonged use with the same resistance; and, if the latter prove satisfactory, it is probable that the regulating screw need not be touched again for a whole cruise, if the buffer be rinsed in lye-water each time after use.

A crank or key, W, is fitted to the squared heads of the regulating and adjusting screws, on which it locks with a spring snap, the latter being operated by the bent arm at one end of the crank. The stud at the other end of the crank is for adjusting the screws J J.

The cast-iron messenger, X, is in two parts, connecting with each other by a dovetail, — or something of similar purpose.

Professor Agassiz and Commander Bartlett added the funnel-shaped trap, and also the leather cushion, Y, around the valve seat, after a preliminary trial with the apparatus.

Working the Apparatus.

It is necessary to first regulate the buffer to cushion the stoppage of the falling weights, which are, cylinder and frame 38 lbs., clamp 4 lbs., messenger 8 lbs., total 50 lbs. The "Blake" adopted a resistance of about 80 lbs. (this resistance being, of course, constant during the whole movement of the buffer), it having been found that a blow of that force resulted in no injury to the apparatus.

On the ascent the buffer must withstand, not only the weight of the fifty pounds of metal, but also the resistance which the water offers to the passage through it of the several parts of the apparatus. Moreover, when the cylinder emerges from the water, it is full of that liquid, and with this increased weight would overcome the stated resistance of the buffer, and force the latter downwards until the lead was reached. To meet these conditions it was not thought advisable to increase the resistance of the buffer, which would involve a heavier blow against the apparatus, but a rope-yarn seizing or stop was placed on the rope about fifteen or twenty feet below the buffer, beyond which the latter could not pass.

Having secured the buffer to the rope about five or six fathoms above

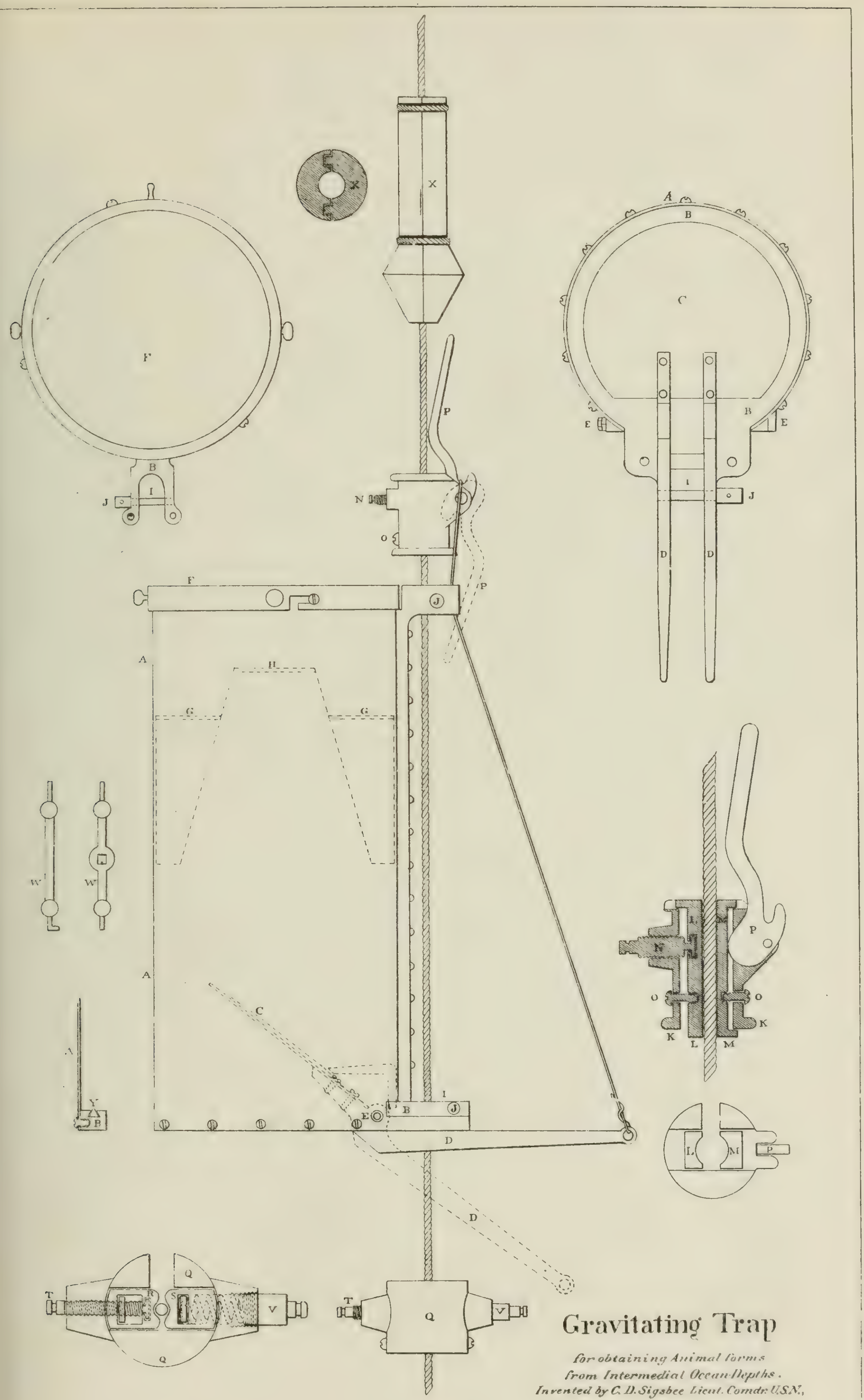
the lead (a very heavy lead to keep the steel rope straight) and payed out the length of rope required to span the stratum to be explored by the cylinder, the clamp and cylinder are attached, the latter being suspended from the former as follows. The rope having been placed between the two sliding chocks of the clamp, the arm of the eccentric tumbler is thrown up, which moves the chock M inwards; then, by means of the adjusting screw, the chock L is pressed against the rope, securing the clamp in position. The cylinder hangs four or five inches below the clamp and is supported by a loop of soft wire which rests on the lip of the tumbler; the ends of the wire, being rove through holes in the upper part of the frame of the cylinder, are fastened permanently to the outer arms of the lever to which the valve is screwed. It is seen that by this method of suspension the weight of the cylinder and its frame is used to keep the valve closed while paying out.* The cylinder should be filled with water, poured down through the upper sieve, to maintain the valve on its seat while the cylinder is being immersed. Rope is then payed out slowly until the cylinder is at the desired depth, when the rope is stoppered, and the messenger sent down.

The messenger strikes the arm of the eccentric tumbler, throwing it down and tripping the cylinder. The tumbler in falling relieves the pressure on the sliding chock M, which is then free to recede from the rope. Messenger, clamp, and cylinder fall together, the valve being held open by the resistance of the water. A current is established through the cylinder, and specimens which enter are retained by the upper sieve. When the buffer is reached, the valve is closed by the pressure against the outer arms of the lever.

A very slight pressure on the adjusting screw of the clamp, after the chocks are bearing against the rope, is enough to prevent the clamp from slipping, but by an increased pressure on the screw a greater force is required to trip the tumbler, and by this feature the arm of the tumbler is utilized to break the force of the blow which the body of the clamp receives from the falling messenger. A few rings of sheet-lead may be laid on top of the clamp and the buffer respectively.

WASHINGTON, D. C., September, 1880.

* It is suggested that, in lieu of the soft wire sling, the friction clamp be constructed to receive the end of a stiff wire rod, proceeding from the ends of the lever D D, and that it be done in such a way that, when the valve is closed and the rod connected with the clamp, the bottom of the latter will be in firm contact with the upper part of the cylinder frame. Such an arrangement would effectually guard against the opening of the valve with any rapidity of descent.



Gravitating Trap

for obtaining Animal forms from Intermedial Ocean-Depths.
Invented by C. D. Sigsbee Lieut. Comdr. U.S.N.,
with improvements
by Alexander Agassiz and Comdr. J. R. Bartlett U.S.N.

No. 10. — *On some Points in the Structure of the Embryonic Zoëa.* *By* WALTER FAXON.

THE embryonic cuticle which clothes the larvæ of the higher *Crustacea* at the time when they leave the egg has been studied with more or less care by Du Cane,* Spence Bate,† Fritz Müller,‡ Gerbe,§ A. Dohrn,|| Stuxberg,¶ Claus,** and P. Mayer.†† Müller first called attention to the fact that the tail of this embryonic skin in certain genera of *Brachyura* (*Achæus*, *Maia*) resembles that of the larvæ of shrimps and prawns, and working upon this hint Mayer has shown the great morphological and phylogenetic value of a careful comparison of the caudal fin of the embryo with that of the following free-swimming stage.

While in Mr. Agassiz's laboratory at Newport, R. I., in the summer of 1879, I made some observations upon the youngest larval stages of a few *Brachyura*, especially *Carcinus mænas* and *Panopeus Sayi*. Although the former species is the subject of Spence Bate's elaborate memoir on the development of Decapod *Crustacea*, I am induced to publish my observations on account of the important discrepancies between them and those of Bate.

Carcinus mænas.‡‡

The young of this species are peculiarly favorable for a study of the embryonic membrane, since it is often retained for twenty-four hours after emerging from the egg. In most species, on the contrary, the first moult takes place within an hour or so after hatching; indeed, in the case of *Gelasimus pugnax* Smith, which I raised from the egg for the express purpose of examining the embryonic cuticle, I have only succeeded

* Ann. Nat. Hist., Vol. III. p. 438, Pl. XI. 1839.

† Phil. Trans. Roy. Soc. London, Vol. CXLVIII. p. 589, Pl. XL. 1859.

‡ Für Darwin, 1864. Eng. Trans. by W. S. Dallas, p. 53. 1869.

§ Comptes Rendus, Vol. LIX. p. 1102. 1864.

|| Zeitschr. Wiss. Zool., Vol. XX. p. 621, Pl. XXX. 1870.

¶ Öfvers. Kongl. Vetensk.-Akad. Förhandl., XXX. (1873), No. 9, p. 6. 1874.

** Untersuchungen zur Erforschung der Genealogischen Grundlage des Crustaceen-Systems, p. 62, Pl. X. Fig. 9. 1876.

†† Jenaische Zeitschr., Vol. XI., p. 246, Pl. XV. 1877.

‡‡ It may not be superfluous to append a list of those who have treated of the development of this common and widely distributed crab: —

in obtaining it by extracting the embryo prematurely from the egg. In this case escape from the egg and the first moult appear to take place simultaneously.

The bursting of the egg-membranes is effected by the convulsive attempts of the imprisoned embryo to extend its abdomen, which is closely applied to the sternum within the egg. The forked tail first extricates itself (Pl. I. Fig. 1), the antennæ then protrude through the breach thus made (Pl. I. Fig. 2), and in a very short time the contortions of the animal have completely torn away the egg envelope. The embryo, swathed in a delicate, perfectly transparent cuticle, now lies on the bottom of the aquarium supinely awaiting its first moult. It is as yet incapable of swimming about and taking food, its only movements consisting of extension and flexion of the abdomen. It is not until the veil is cast off that the animal loses its embryonic character, and assumes the part of an active, free-swimming larva, with mouth parts adapted for seizing prey.

On issuing from the egg, the young measures $\frac{1}{4}$ mm. in length (Pl. I. Fig. 3). Within the transparent cuticle the zoëa may be distinctly seen as it will emerge on the first moult. The cuticle is not conformable to the underlying larval integument, as it has neither dorsal nor frontal horns, and the antennæ and tail are very different. The carapace does not at first extend far enough back to cover the base of the swimming-feet, so that the abdomen appears much longer relatively than it does a short time after hatching.

At the joints between the segments of the abdomen of the zoëa the cuticle does not follow the indentations, but otherwise rests conformably upon it. The two prongs of the forked tail of the zoëa are compressed into a very small space by means of a complex folding produced by an invagination of the middle third of the prongs, which does not involve

J. V. Thompson, Phil. Trans., 1835, p. 359, Pl. V.

Heinrich Rathke, Zur Morphologie, p. 97. 1837.

C. Du Cane, Ann. Nat. Hist., Vol. III. p. 438, Pl. XI. 1839.

H. D. S. Goodsir, Edinburgh New Phil. Jour., Vol. XXXIII. p. 181, Pl. III. 1842.

M. P. Erdl, Entwicklung des Hummereies, p. 27, Pl. II. 1843.

R. Q. Couch, "Ann. Rep. and Trans. Roy. Cornwall Polytechnic Soc. for 1843." (I have not seen this memoir. Some account of it is given in Bell's History of the British Stalk-eyed Crustacea, Introduction, pp. xlix. – liv., Figs. *c*, *d*, *e*, and pp. 79 – 81. 1853.)

C. Spence Bate, Phil. Trans., Vol. CXLVIII. p. 589, Pl. XL.–XLVI. 1859.

V. Hensen, Zeitschr. Wiss. Zool., Vol. XIII. pp. 340, 362, Pl. XX. Fig. 25. 1863. (Auditory organ of the young.)

Anton Stuxberg, Öfvers. Kongl. Vetensk.-Akad. Förhandl., XXX. (1873), No. 9, p. 7. 1874.

the distal third which lies within the invaginated portion like a sword within its sheath. The same thing is seen in the spines which are found on each border of the caudal prongs (Pl. I. Figs. 6, 7, 12).* The tail of the embryo has an entirely different form. Each half of the fork is produced into seven long spines (Pl. I. Fig. 7). Of these, the three inner correspond to the three internal spines on the tail of the zoëa (Pl. II. Fig. 2). The fourth is the homologue of the prong itself, while the fifth, sixth, and seventh answer to the three minute ones (Pl. II. Figs. 2, 5, 6, 7), which are situated on the outer side of the fork. Curiously enough, the spines of the two stages tend to an inverse proportion, the fourth, or smallest in the embryo, being homologous with the prong of the zoëa tail, while the fifth, or largest, is replaced by one of the small external spines (5′) in the subsequent stage. The fourth and seventh are naked; the rest are fringed with delicate hairs. In a few instances I found the spines of the embryonic skin invaginated in the way already described in the case of the spines of the caudal fin of the zoëa. In one example this invagination affected the second, third, and fifth spines (counting from the inside), (Pl. I. Fig. 6,) in another the third and fifth, in another the third only. Without doubt all the longer spines are thus invaginated within the egg.†

The two pairs of antennæ of the embryo, again, have a much greater development than in the zoëa, exceeding in length the swimming-feet, and reaching, when stretched backwards, beyond the base of the abdomen (Pl. I. Fig. 3). The first pair (Pl. I. Fig. 4) consists of a basal segment, within which lies the antennule of the zoëa, and which bears two branches, viz. a long one furnished with three longitudinal rows of fine setæ, and a very short one.

The second antennæ (Pl. I. Fig. 5) divide a short distance from the base into two branches, one of which has the form of a simple, blunt, finger-like process (*a*); the other divides again into three branches (1, 2, 3), which are fringed with delicate hairs. In some specimens, at the moment of issuing from the egg, one or more of these branches is infolded like an inverted glove-finger. The short and blunt process (*a*) encloses the spinous process (Spence Bate) of the antenna of the zoëa, while the triple branch (*b*), which forms the bulk of the antenna of the embryo, has its homologue in the external branch, or scale (*squamiform*

* According to Milne Edwards, the hairs of the new test of adult crabs which are about to moult are invaginated in a similar way. (Histoire Naturelle des Crustacés, Vol. I. p. 55. 1834.)

† Cf. Goodsir, *op. cit.*, Pl. III. Fig. 17; Claus, *op. cit.*, Pl. X. Fig. 9.

appendage of Spence Bate), of the enclosed zoëa. The flagellum of the antenna of the adult, seen in the first zoëa stage as a small protuberance (Pl. I. Fig. 10, *c*), has no representative in the embryonic antenna. The spinous process and scale of the zoëa antenna are much shortened by invagination, like the structures of the tail already described.*

Morphology of the Antennæ. — One can hardly avoid the conclusion that, in the same way that the seven-spined forked tail of the embryo is a reminiscence of the *Gabelschwantz* (P. Mayer) of the primitive Decapod, so the greatly developed, setiferous antennæ are an inheritance from ancestors in which these appendages subserved locomotive functions, as in the *Nauplius.* The typical second antenna of the *Zoëa* consists of a basal stem produced at its distal end into a long serrate spine (Pl. I. Fig. 10, *a*; Pl. II. Fig. 3, ii. *a*), and bearing besides an articulated squamiform appendage (*b*). The spine is seen in a rudimentary form in the larvæ of the shrimps, prawns, and *Paguridæ.* The squamiform appendage is homologous with the external branch of the second antenna of the larval *Macroura*, and with the antennal "scale" of the adult *Macroura.* Both the spinous process and the squamiform appendage become aborted in the development of the *Brachyura.* The flagellum of the second pair of antennæ of the adult crab is wanting in the youngest zoëa stages, or is represented by a small papilla merely (*c*).

If the relation of the embryonic antenna to the *Nauplius* antenna, suggested above, be correct, it follows that the bulk of the antenna of the *Nauplius* is not represented by any homologous part in the permanent antenna of the crab. If, on the contrary, it be claimed that the large fringed lobes of the embryonic antennæ simply represent antennal setæ, they still point back to a primitive condition in which the first two pairs of appendages were provided with *Schwimborsten*, and served as natatory organs.

The labrum, mandibles, metastoma, and maxillæ have nearly the same form which they have in the zoëa stage which follows. The long swimming-setæ of the first and second maxillipeds, which play so conspicuous a part in the life of the zoëa, are very much shortened by invagination, and entirely covered by the embryonic cuticle.

The third pair of maxillipeds and the two following pairs of appendages of the zoëa show through the transparent membrane as three pairs of small buds (Pl. I. Fig. 3, viii., ix., x.), but there are no corresponding structures in the embryo.

* A. Dohrn, who observed similarly formed antennæ in the embryo of a species of *Portunus* (*l. c.*), has confounded the two pairs.

The young remains in this embryonic condition for about twenty-four hours (at least in confinement). In the mean while it has increased in size to such a degree that the delicate investing membrane is no longer ample enough for the enclosed zoëa and the first exuviation takes place. The cuticle of the abdomen is cast first, commonly coming off in one piece (Pl. I. Fig. 9, 9′). The dorsal spine, which has been invaginated like the parts already described, and laid forward over the back, begins to be evaginated, and to erect itself, and thus aids in splitting the membrane along the back. The rostrum, which has been applied to the breast, also emerges, and the abdomen, freed from the embryonic cuticle, is now used to clear the appendages of the cephalo-thorax, in this wise: the ends of the two prongs of the tail-fork are bent so as to form minute hooks (Pl. I. Fig. 12): when the abdomen is flexed, these little hooks catch in the membrane covering the cephalo-thoracic appendages, and on extending the abdomen again the membrane is torn off (Pl. I. Fig. 9).

The dorsal horn is commonly evaginated, and assumes its position with a slight backward curve even before the embryonic skin is entirely got rid of. In specimens which have just cast the embryonic skin, a break in the trend of the spine indicates the rim of the former invagination (Pl. I. Fig. 14). The rostral spine now projects downward at a right angle with the long axis of the body. The setæ on the various parts of the body unroll themselves, the mouth parts become functional jaws, enabling the young animal to feed; the two pairs of swimming-feet, provided each with four long swimming-setæ on their external branches, become active agents for locomotion, and now, in place of the inert and pupa-like embryo, we have a vigorous free-swimming larva.

Besides the great difference between the two stages caused by the sudden development of the dorsal and frontal spines, the two pairs of antennæ and the tail have an entirely different form. Both pairs of antennæ are now of relatively small size. Those of the first pair are composed of but one segment, which carries three long sensory threads at the tip. This segment corresponds to the basal segment of the first antenna of the embryonic stage.

The second pair of antennæ consist of a basal piece with a long serrate spinous process (Pl. I. Fig. 10, *a*; Pl. II. Fig. 3, *a*), which lies in the short, blunt process of the antenna of the embryonic stage (Pl. I. Fig. 5, *a*), and a short, blunt protuberance (Pl. I. Fig. 10, *c*), the rudiment of the antenna of the adult crab.

In addition to these processes, there is articulated to the basal piece

a long joint with a long and a short hair on its extremity (Pl. I. Fig. 10, *b*; Pl. II. Fig. 3, *b*). This is the homologue of the "scale" of the antenna in *Macroura*, and appears to represent the main, triple portion of the embryonic antenna (Pl. I. Fig. 10, *b*).

The tail (Pl. II. Fig. 2) has now the form so characteristic of the zoëa of *Brachyura*. It is a forked piece, each prong of the fork bearing three setæ on the inner side near the base, and three minute ones on the outer side. The prongs of this forked tail themselves are homologous with the fourth spine of the embryo tail, as before pointed out. The outer three (5, 6, 7) diminish in size successively.

Although I succeeded in keeping some of these zoëæ alive for seven days, none passed through another moult.

In Spence Bate's classic memoir on the development of *Carcinus mœnas*, the embryonic membrane which covers the zoëa when it first quits the egg is described and figured as conformable to the whole animal, the tail and antennæ not excepted. Thus are ignored the most interesting and suggestive structural features of the embryo. This error of observation is the more remarkable, since the structures in question were figured with approximate accuracy twenty years before by that close observer, Captain Du Cane.*

H. D. S. Goodsir† also seems to have seen the same structures, although his description and figures are very incorrect. The "curious brush-shaped appendages of the embryo," which "drop off when the animal has escaped from the ovum, and are replaced by spines,"‡ are evidently the invaginated caudal spines of the embryonic cuticle, such as are represented in our Plate I. Fig. 6. Spence Bate's identification of the two pairs of swimming-feet of the zoëa with the second and third pairs of maxillipeds of the adult, instead of with the first and second pairs, was not so strange; but why does he persist in the old error, even in his latest papers,§ after it has been particularly pointed out by Fritz Müller,‖ Stuxberg,¶ Claus,** and others?

* *Op. cit.*, Pl. XI. Figs. 1, 5.

† Edinburgh New Philosoph. Jour., Vol. XXXIII. p. 182, Pl. III. Figs. 16, 17. 1842.

‡ Pp. 182, 191, Pl. III. Fig. 17.

§ Report on the Present State of our Knowledge of the Crustacea. Rep. Brit. Assoc. Adv. Sci., 1875, p. 48; 1876, p. 89; 1877, p. 44; 1878, pp. 7, 8.

‖ *Op. cit.*, Eng. Trans., p. 52.

¶ *Op. cit.*, p. 10.

** Würzb. naturw. Zeitschr., 1861, p. 30. Untersuchungen zur Erforschung der Genealogischen Grundlage des Crustaceen-Systems, p. 62. 1876.

Panopeus Sayi.

The remarkable zoëa represented on Plate II. Fig. 4, a very common form on the southern shore of New England, I raised from the eggs of *Panopeus Sayi* in the summer of 1876. It differs strikingly from all other zoëæ with which I am acquainted in the structure of the second pair of antennæ (II.), which consist of a single monstrously developed spine equal in length to the rostrum. In other regards the zoëa is not specially noteworthy. The carapace has, in addition to the rostral and dorsal spines, a pair of short lateral spines. In the middle line of the back, well forward toward the eyes, is a well-marked hump.

The caudal fork (Fig. 5) bears but four pairs of spines; the two exterior pairs (6 and 7 in *Carcinus*) are wholly wanting.

To which part of the typical second antenna of the zoëa, as described on page 162, does the long, rod-like antenna in this species correspond? In order to answer this question we must examine the cuticle of the embryo. This is represented by Fig. 8 of the plate. It has a form similar to that previously described in *Carcinus mænas* (Pl. I. Fig. 5); but here the branch marked 3 is split nearly to the base, making an apparently quadruple structure in place of the triple branch of *Carcinus*. The blunt, finger-like process (*a*) encloses the antenna of the zoëa (*a′*), which is marvellously shortened by evagination. The homology of the zoëa antenna in this case is thus fixed. It represents the spine of the normal antenna.

The cuticle covering the first pair of antennæ (Fig. 7) has the same parts as the corresponding structure in *Carcinus*, and the same with the tail (Fig. 6), in which the two external spines (6 and 7), which are entirely wanting in the first stage of the zoëa, are well developed.

CAMBRIDGE, July, 1880.

EXPLANATION OF THE PLATES.

PLATE I. **Carcinus mænas.**

Fig. 1. Embryo beginning to emerge from the egg.
Fig. 2. The same, a little further along.
Fig. 3. Embryo shortly after hatching.
Fig. 4. First antenna of the same.
Fig. 5. Second antenna of the same. The branch (3) invaginated : *a′*, spine of the antenna of the zoëa seen through the cuticle ; *b′*, squamiform appendage of the antenna of the zoëa.
Fig. 6. Tail of the same : the enclosed tail of the zoëa is shaded ; spines 2, 3, and 5 are invaginated.
Fig. 7. The same : all the spines of the embryonic tail evaginated.
Fig. 8. Invaginated rostrum of the zoëa, as seen through the embryonic cuticle.
Fig. 9. Young in the act of exuviating the embryonic cuticle.
Fig. 9′. The cuticle of the abdomen, just cast from Fig. 9.
Fig. 10. Second antenna of the zoëa : *a*, spine ; *b*, squamiform appendage ; *c*, rudiment of the flagellum of the adult. The spine and squamiform appendage are still invaginated.
Fig. 11. To show the way the dorsal spine lies at the time of the first moult. It has become evaginated, but not yet erected.
Fig. 12. Extremity of a prong of the caudal fork, to show the unfolding of the distal part, and the terminal hook.
Fig. 13. Rostrum and antennæ of a zoëa at the moment of exuviating the embryonic cuticle.
Fig. 14. Dorsal spine of zoëa immediately after casting the embryo skin. The break near the middle of the spine shows the rim of the invagination during the earlier period.

PLATE II.

Figs. 1–3. **Carcinus mænas.**

Fig. 1. First stage of the zoëa. The appendages are marked by consecutive Roman numerals.
Fig. 2. Tail of the same.
Fig. 3. First and second antennæ of the same.

Figs. 4–10. **Panopeus Sayi.**

Fig. 4. First stage of the zoëa.
Fig. 5. The same, viewed from behind.
Fig. 6. Tail, with the embryonic cuticle.
Fig. 7. First antenna of the embryo.
Fig. 8. Second antenna of the embryo : *a′*, antenna of the zoëa seen through the embryonic antenna.
Fig. 9. Second maxilla of embryo. The shaded part represents the appendage of the zoëa within.
Fig. 10. End of swimming-branch of first maxilliped. The long swimming-setæ are shortened by invagination and closely invested by the embryonic cuticle.
Fig. 11. Tail of *Gelasimus pugnax*, Smith, first stage of the zoëa. Spines 5, 6, 7, are entirely wanting.

No. 11.—*New Species of Selachians in the Museum Collection.* *By* Samuel Garman.

Scyllium ventriosum n. sp.

Body very stout in the anterior half, hinder portion slender. Head flattened, as broad as long. Snout short, blunt. Eyes medium. Spiracle small. Nostrils near the mouth, separated by a space equal to the length of the snout, with a valve on each side. Anterior nasal valve short, broad, more than half as wide as the nostril, reaching the teeth; posterior smaller, of similar shape and hidden by the first. The distance of the valves from each other is equal to three fourths of the length of the snout. Mouth wide, crescent-shaped. Labial folds rudimentary, not visible when the mouth is closed. Teeth small, central cusp long and slender, with two lateral cusps on each side, the outer of which is feebly developed, in fifty-four rows in the upper jaw. The symphysis bears no teeth; on each side of it the first two rows are very small. Gill openings narrow, the fourth and fifth over the pectoral, the third twice the width of the fifth. Pectorals broad and short; margins convex, the anterior one fourth longer than the posterior; angles rounded. Ventrals short, margins convex, outer extremity broadly curved, posterior blunted. First dorsal twice the size of the second, base above the posterior half of the ventral, height little less than the length of the base, borders convex, upper extremity round, posterior blunt. Second dorsal smaller than the anal, distant from the first the length of the posterior border, its entire length less than that of the base of the anal, upper border curved, posterior straight, hinder angle acute. Tail less than a fourth of the total, its width contained in its length two and a half times, notched near the extremity on the lower side, no pit at the root. The shape of the tail is similar to that of *S. stellare* or *S. canicula*, though broader than that of either. Scales pedicellate, sharp and coarse. Nine circuits in the spiral of the intestine.

Color grayish brown, spotted and banded with darker. The spots are indistinctly outlined, irregular in size and position. Bands transverse, twelve or more in number; five of them occur between the eyes and first dorsal. Lower surface darker, olivaceous and more uniform. The specimen, an adult female, is twenty-nine inches in length, and measures fifteen inches around the body between dorsal and pectorals.

This species differs from *S. chilense* in the nasal valves, labial folds, lateral cusps on the teeth, small second dorsal and its position with respect to the anal, and the numerous transverse bands. One specimen from Valparaiso.

Rhinobatus lentiginosus n. sp.

Outlines of body and fins similar to those of *Horkelii* and *undulatus*. Rostral cartilage long and narrow, a small groove near the head; ridges close together from base to extremity. Eyes large. Spiracles half as large as the eyes, with two folds. Head narrow, concave between the eyes. The width of the interocular space equals that of the nostrils or their distance apart. Half the length of the snout is less than the distance between the outer angles of the nostrils. Mouth nearly straight, a little less than twice the width of the head between the eyes. Scales small, smooth. Spines of the dorsal series and the three in front of each eye very small; those above the eye and spiracle not noticeable. No larger spines on shoulders or rostrum. The largest spines on the body are a group of five on the top of the end of the snout.

Color a light grayish-brown freckled with small spots of lighter; uniform brownish below. On the lower side of the snout there are faint indications of markings similar to those of *undulatus*.

Distinguished from *Horkelii* and *undulatus* by the colors, the horn-like spines on the end of the snout, the absence of spines on the shoulders, the narrowness of the head as compared with the width of the mouth, the shorter distance from snout to mouth, and the greater distance from mouth to vent. Total length 22.9, snout to mouth 4.1., snout to vent 9.9, and width of pectorals 7.4 inches.

An adult female secured in Florida by Prof. L. Agassiz.

Rhinobatus planiceps n. sp.

Disk, including ventrals, rhombic, about one and a half times as long as wide. Anterior borders of pectorals straight, more than twice as long as the convex posterior margins. Angles of pectorals rounded, the hinder not extending farther than to the vent. Outer angle of ventrals rounded, posterior acute. Head broad, flat. Rostral cartilage medium, dilated at the extremity, with the ridges close together in the anterior third of their length. Snout rather broad, with rounded extremity. Eyes moderate. Spiracle immediately behind the eye, smaller than the orbit, with a single fold on the posterior side. Anterior nasal valve not dilated; posterior two-lobed. Mouth nearly straight. Body covered with shagreen above and below. Tail much depressed, with a fold on each side. Second dorsal distant from the caudal the length of its base. Bases of the dorsals distant from each other the length of the anterior borders. Scales larger over the central portions of the disk. Compressed hooked spines in a median row on back and tail, in two patches on each shoulder, and a series above each eye. On the young these spines are much more prominent and regular in size than on the adult.

Color brown, light between and on each side of the rostral ridges; white below. Young specimens with a number of small round white spots on each side of the dorsum.

The following measurements are taken from a young male: —

Total length	19.0 inches.
Snout to end of ventrals	10.2 "
Snout to vent	8.0 "
Snout to mouth	3.5 "
Width of pectorals	6.9 "

Twenty-one specimens from Payta, Callao, and Galapagos Islands, collected by the Hassler Expedition.

Trigonorhina alveata n. sp.

Disk, including the ventrals, rhombic, longer than wide. Anterior borders of pectorals nearly straight; posterior convex. Snout truncated, as wide on the end as the space between the eyes. Rostral cartilage wide and strong, deeply grooved on its upper surface. Rostral ridges prominent, widely separated, nearly or quite parallel from base to extremity. Spiracles large, equal in diameter to the orbit, without a fold on the side. Fin angles rounded, with the exception of the obtuse posterior angles of the dorsals. Dorsals elevated, behind the ventrals; the length of the base of the first less than the length of its posterior border; base of the second equal to its posterior margin. The base of the first is equal to its distance from the ventral or the second dorsal. Anterior nasal valves dilated, continued beyond the inner angles of the nostrils, but separated from each other by an interspace; posterior two-lobed. Anterior extremities of the pectorals widely separated from the rostral cartilage, extending very little in advance of the eyes. Mouth in a low arch, regularly curved from the corners. Teeth small, blunt, in a hundred and ten series in the upper jaw. Claspers long, slender, knobbed at the end. Tail with a thick fold on each side. Caudal fin rounded, without indentation. Back thickly covered with small scales, among which are scattered larger ones. A median row of large blunt tubercles on back and tail, and two short rows parallel to this on each shoulder. The bases of the tubercles are so covered by the skin and small scales that they appear as rounded prominences with a small spine on the summit.

Color grayish brown. Near the extremities of the rostral ridges there is a band of dark brown; between this and another dark band which crosses the bases of the ridges there is a light band. A dark band across the head between the eyes is somewhat confluent with the band in front of it, which makes the fore part of the head dark, but leaves the prominences in front of the eyes light-colored. The remainder of the upper surface is more or less clouded by faint indications of transverse bands. These are probably distinct in the young. With the exception of a dark spot on the posterior angle of each pectoral, the lower surface is white.

Total length	33.4 inches.
Snout to end of ventrals	18.0 "
Width of pectorals	15.5 "
Snout to vent	13.3 "
Snout to mouth	4.1 "
Width of mouth	2.5 "
Distance between outer angles of nostrils . .	3.2 "

Trigonorhina exasperata.

This is the species described by Jordan and Gilbert under the name *Platyrhina exasperata*, and from which at a later date these authors drew the characters for the genus *Zapteryx*. The latter does not seem to differ from the genus *Trigonorhina* of Müller and Henle. The species *T. exasperata* and *T. alveata* are closely allied.

The genus *Platyrhina* is closely related to *Trigonorhina*, and with it belongs to the family *Rhinobatidæ*. Both genera have broad based tubercles in a vertebral series and on the shoulders. *Sympterygia* and *Platyrhina* have little or no affinity for each other. Of the *Rhinobatidæ* the latter is, perhaps, the nearest approach to the *Rajæ*. It is out of place with the *Rajidæ*, as located by Dumeril and Günther.

Trygon lata n. sp.

Disk quadrangular, one fourth wider than long. Anterior margins nearly straight, forming a very blunt angle at the snout, rounded near the outer extremities; posterior convex; inner straight a portion of their length. Ventrals truncate, rounded. Snout produced, forming a rounded prominence in front of the margins of the disk; length from forehead less than the width of the head. A line joining the wider portions of the disk passes nearer to the head than to the shoulders. Tail more than twice as long as the body, subcylindrical, without a trace of keel above, roughened with small tubercles, with an irregular series of broad-based conical tubercles on each side; a long narrow cutaneous expansion below has its origin opposite that of the spine, and terminates in a keel which continues to the extremity. A pair of large compressed erect tubercles stand immediately in front of the caudal spine, and a single one is placed over the middle of the pelvic arch; these suggest a continuous series in larger specimens. Three larger elongated tubercles with points directed backward — similar to those of *hastata* — occupy the middle of the shoulder girdle. Mouth curved, six (5–6?) papillæ at the bottom; two of these are in the middle in front where usually there is but one.

Color light olive, probably greenish in life, white below. Distinguished from *T. centrura* by the prominent snout, the shape of the tubercles on the middle of the back, and the narrowness of the posterior portion of the disk.

Length of body 16, length of tail 35.3, and width of pectorals 20.5 inches. Collected at the Sandwich Islands by Andrew Garrett.

Trygon longa n. sp.

Disk quadrangular, about one sixth wider than long. Margins nearly straight, anterior meeting in a blunt angle on the end of the snout. Outer angles rounded, posterior blunt. Ventrals rounded. Tail more than twice as long as the body, roughened with small asperities, depressed anteriorly, compressed behind the spine, keeled above the compressed portion, with a long narrow cutaneous expansion on the lower side. Mouth curved, with five

papillæ. A row of small tubercles behind the head on the shoulder girdle. It is likely that large specimens are provided with tubercles on back and tail.

Distinguished from *T. lata* by the shape of the disk and snout, and the keel on the tail; from *T. centrura* by the straight margins of the pectorals and the keel.

One specimen secured at Acapulco, Mexico, by Prof. Alex. Agassiz. One light-colored, reddish-brown specimen from Panama, by the Hassler Expedition.

Length of body 11.5, length of tail 28, and width of pectorals 13.8 inches. Length of body of second specimen 9.3, length of tail 24.5, and width of pectorals 11.2 inches.

Trygon brevis n. sp.

Disk quadrangular, a little wider than long. Anterior margins nearly straight, curved near the outer extremities to meet the convex posteriors, meeting in a blunt angle on the end of the snout. Outer and posterior extremities of pectorals round, without trace of angles. Ventrals broad, truncate, with angles rounded. Tail less than one and a half times the length of the disk, tapering to an acute point, depressed as far as to the spine, thence compressed to the end of the cutaneous fold and round from this point to the end, with a short elevated membranous expansion behind the spine, and a longer and wider one on the lower side extending below the former and the spine. The expansions have their hinder extremities opposed; they end quite abruptly, and are widest near the termination. Mouth with five papillæ, outer small. Teeth small, blunt. Upper jaw indented in the middle; lower, with a prominence in front. Disk naked in the young. Adult specimens have three rows of tubercles on the middle of the back disposed as are those of *T. hastata.* A large specimen from Payta has three large, erect, broad-based tubercles in front of the caudal spine, and the tail rough with smaller ones. The short rows on the shoulders contain from one to four, and probably increase in number with age, as is the case with closely allied species from the Atlantic coast.

Color olive or grayish brown, reddish near the edges; below white, with round spots of brownish under the base of the tail.

Compared with *hastata* this species differs in the shorter tail, the rounder extremities of the disk, and the shape and size of the tubercles and membranous fins.

T. hastata has no expansion on the top of the tail, and that on the lower side is very long, of moderate width, and tapers gradually. Those of *brevis* are comparatively short and broad; they rise gradually and terminate abruptly.

From *T. Sayi* this species is to be distinguished by the great development of the caudal expansions, their shape and length, and by the tubercles on shoulders and tail. A large female measured in length of body 17, length of tail 23, and width of pectorals 18 inches; a young male, length of body 8.1, length of tail 12, width of pectorals 9.2 inches.

Including this and the preceding, the number of American species properly belonging to the genus Trygon is increased to seven.

Trygon brachyurus and *T. reticulatus*, recently described by Dr. Günther, belong to the genus *Potamotrygon*. The species redescribed and figured by Dr. Steindachner in 1878 as *Tæniura magdalenæ* also belongs to that genus. It needs but a slight knowledge of the anatomical differences existing between the *Potamotrygones* and the *Tæniuræ* proper to convince any one that both cannot be retained in the genus *Tæniura*.

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CONTENTS.

PART II.

No. 12. — *Maturation, Fecundation, and Segmentation of Limax campestris*, Binney. *By* E. L. Mark.*

The observations of the past five years † on the earliest stages in ontogeny have contributed more to the solid advancement of biological knowledge, than those of any corresponding interval since the studies of Max Schultze and others paved the way to a science of Biology.

More refined methods of research have resulted in more exact knowledge of phenomena. A closer study of details has opened the way to a broader comprehension of their significance.

It was with the hope of adding something to the empirical acquisitions in this field, that I undertook the studies whose results follow.

A. OBSERVATIONS.

The eggs of *Limax campestris*, Binney, are found in moist places, protected from the drying influences of direct sunlight and currents of air. They are frequently met with in the vicinity of a small stream, some stagnant pool, or in low meadow-land. Open woodland presents favorable conditions for their development, affording sufficient warmth, and preventing too direct sunlight and constant winds. In such locations loose piles of decaying wood are often chosen for the deposit of eggs. At other times, when the ground is less protected, they may be found under loose stones, or even in the bed of some spring-time water channel, where crevices in clumps of earth afford protection. The most of the material which I have studied was obtained from slugs kept in confinement. These were collected from partially shaded ground with scanty grass-growth, in the vicinity of Fredonia, N. Y. A portion, however, were from low grass-land near the Museum of Comparative Zoölogy in Cambridge.

* I desire to acknowledge my indebtedness for the use of books to the extensive libraries connected with the University, as well as to the Boston Society of Natural History, the Boston Public Library, and the Boston Medical Library. I am under obligations to the librarians of all these institutions for personal or official favors, for which I take this opportunity of expressing my thanks.

† The unfortunate delays which have attended the publication of this paper are liable to mislead the reader, unless it is borne in mind that it was prepared early in 1879. See a preliminary notice in Zoöl. Anzeiger, 2 Jahrg., p. 493.

The eggs are usually found in clusters of about a dozen each, though the number is subject to considerable variation. Sometimes they are only loosely collected together, or even moderately scattered over an area of a few inches; at others, they are closely packed in a more or less rounded mass. Owing to the nature of the place chosen for deposit, they are often arranged in rows, as in the narrow cracks of moist, decaying wood, or in the chinks in cakes of earth. On splitting the wood or breaking open the earth, they are occasionally found to fill all the available space completely, and if the cavity is broad and shallow they are accordingly arranged in rows a single layer deep. It is wonderful into what narrow crevices the eggs are sometimes crowded, apparently for better protection from enemies.

They vary in external appearance according to the hygrometric conditions in which they are found. If the requisite amount of moisture is available, they are of a full, plump outline, and resemble beads of pearl or frosted silver. If their surroundings are dry, they have a shrivelled look, are more or less flattened, and have a faint yellowish tint. Moisture restores them to their normal shape and color.

A careful examination will show some modifications of form, especially if the eggs are taken from different groups. Those taken from a single mass are usually rather uniform in appearance, though they may show noticeable differences. The same is true of their dimensions. The average long diameter is a little more than 2 mm. The short diameter may almost equal the long diameter, or it may be hardly more than half as long.

Sections of eggs at right angles to the long axis are almost circular, and never differ in any constant manner from that form; but sections coinciding with the long axis would, in most cases, show oval outlines varying, as has been indicated, in the proportions of their axes. The curvature at either end of this oval is usually about the same, although in some cases one is more pointed than the other. Furthermore, one end (sometimes both) may be drawn out into a sort of cue, which varies greatly in different eggs. Rarely the cues of a couple are continuous. In those found at Fredonia I have never seen more than two thus united. Usually all are quite separate, even though lying in groups close together. In those found at Cambridge I have observed a greater tendency to this union, and once counted thirty thus joined into a delicate rosary. There could be no doubt in this case that all were laid by the same individual, and in regular succession. Indirect evidence that those contained in a given mass were also all laid at the

same time (i. e. in rapid succession) is usually to be found in the comparative uniformity in size and shape which the individual eggs of the group present. The greatest number observed which thus indirectly gave evidence of belonging to a single deposit was thirty-seven ; on the other hand, it is quite certain that a very small number may be deposited at one time by slugs that are held in confinement. That the same is true of unconfined animals, I can only infer from finding, now and then, small groups or single eggs far removed from any others.

The external surface of the egg is not smooth, but raised in almost inperceptible bosses, which give to it the frosted look mentioned. Immersed in water and examined under a low power of the microscope, it is seen to be composed of a central, slightly yellowish, homogeneous portion, much more nearly spherical than the whole egg, and of two thick coats of investment, which are colorless, and give it rigidity and a great degree of elasticity. The central mass occupies from two thirds to four fifths of the diameter of the whole egg. The two enveloping layers are not of uniform thickness, and may become at one or both ends exceedingly thin. The outer layer is composed of colorless laminæ, which are for the most part nearly concentric, although at intervals they may become thinner and disappear. The cue is formed from this layer alone. There may often be distinguished a half-dozen principal laminæ, and also, with a higher magnifying power, secondary laminæ of varying thickness, and often in large numbers. The inner layer is likewise colorless and transparent, but shows no trace of lamination. When the outer layer is cut, it is found to be resistent and elastic; but the inner layer offers less resistance and is rather viscid. The inner layer is separated from the central, yellowish mass by a very firm, structureless membrane, which exhibits a great tendency to wrinkle, especially when moisture is withdrawn from the egg. This firm structure is the *membrana albuminis.* The contained yellowish substance is viscid, like the white of the hen's egg, and like it is albuminous. In the freshly laid egg it appears quite homogeneous. It is called the albuminous envelope, but from its great abundance here its nature as an envelope is not striking. In this substance are suspended two structures which are conspicuous in all freshly laid eggs when examined with a sufficiently high power. One of these is tortuous, and usually extends from near the surface of the albumen to the vicinity of the other structure. It resembles an irregularly twisted, or here and there constricted, thin-walled tube. As I hope to make it the object of further study hereafter, I will only add that it has been compared to the chalaza

of the hen's egg, and will pass to the consideration of the other body, — the *vitellus*, or egg proper, — to which all the other parts are simply accessories. This vitellus, or yolk, has not more than one twentieth the diameter of the whole egg, and when the latter is freshly laid it appears as a minute speck, 125 μ. in diameter, just visible to the unaided eye as a whitish dot, which usually has an eccentric position. It is to the study of the yolk, and the changes it undergoes, that I shall confine my attention.

Some of these changes may be followed in the living egg under the microscope ; other and remarkable changes, which up to within a few years had escaped the attention of embryologists, are meanwhile going on within the yolk, and are either altogether hidden, or are only partially visible to one studying the living specimen. It is only by the use of certain acid reagents, which have the immediate effect of killing the egg, and at the same time of hardening it, that these internal conditions may be successfully studied. In considering the successive metamorphoses which the yolk undergoes, it will perhaps be best to follow the course which the observer is compelled to take ; that is, to notice first what may be observed in the living egg, and then to supplement the knowledge thus gained by such instantaneous pictures as the hardening process affords. The more numerous these views, and the more frequent and regular the intervals at which they are taken, the more complete will be the data for interpreting these indirectly observed phenomena.

The changes which it is proposed to follow in this paper are only such as occur between the time the eggs are excluded and the end of the first segmentation. Inasmuch as the following observations begin with the deposited egg, — i. e. do not include a study of the ovarian egg, nor of any of the changes it undergoes within the body of the parent, — I cannot claim for them the completeness I wish they possessed. Recent studies on the very early stages of eggs of other animals will, however, enable us to make a better use of these limited observations than could be made otherwise. For the time indicated, I trust they will be found tolerably complete and connected.

The nature of the phenomena which transpire within the limits of the time selected allow one to group the observations about three principal heads : —

1. The changes connected with the ripening of the egg.
2. Fecundation of the mature egg.
3. Segmentation, or cleavage.

The observations under the first will be least complete, because they

commence after a part of these maturation changes have already transpired. These three series of events will be treated in the order mentioned, as that is substantially the order of their occurrence in time; it should be mentioned, however, that they are not strictly and completely consecutive, for each series of changes is still incomplete at the beginning of the next following, — there is, as it were, an overlapping in time, — and for this reason it will be less advisable to follow the strict chronological, than the physiological order as above indicated.

As far as regards what may be observed on the living egg, the very accurate studies of Nicholas Warneck, though made as long ago as 1850, leave very little room for additions.

It was some time after my first studies (middle April — middle May, 1877) on Limax were ended before the opportunity was afforded for an examination of the literature on the development of pulmonates, which was entirely inaccessible to me at the time I was making the observations. Among other references in Bronn's *Die Klassen und Ordnungen des Thier-Reichs* was that which first directed my attention to this valuable paper by Warneck, hitherto unknown to me even by title. I was temporarily deterred from publishing my studies by the fact that this observer had already published so truthful and complete an account of the development in the case of a slug very nearly related to the one which had formed the basis of my investigations. There were still some points (more especially in the stages of segmentation, which will fall outside the limits of the present paper) in which my observations were at variance with those of the Russian naturalist. It was in part these matters of disagreement, but more especially the influence of the recent writings of Bütschli, Hertwig, Fol, and others, which determined me, early in 1878, to renew my observations at the first opportunity, and to address particular attention to the phenomena to be observed before and during the first segmentation. I was able to devote only a few days to this study in Cambridge, during the latter part of June. The most of the observations were made at Fredonia, in August, 1878.

I. Maturation.

In eggs examined directly after their deposit the vitellus appears as a spherical mass of a slightly yellowish or brownish tint, with perfectly clear, sharp outline, about 0.125 mm. in diameter. It has greater density than the surrounding albumen. Its opacity is occasioned by an immense number of granulations, varying in size. Part of these

promptly swell when, by rupture of the yolk, they are brought into contact with water, and assume spherical contours, with delicate outlines and diameters varying from 2 to 8 μ. Others remain small and of greater refractive power, under the same circumstances. They seldom exceed a fraction of a micro-millimeter (μ) in diameter. (Fig. 26.) These granules are held in suspension by a viscid transparent protoplasm. Their distribution is not always uniform, so that an irregular cloudy appearance often characterizes the yolk. In any optical section the peripheral portions of the sphere, owing to the diminished quantity of these granules which the light is compelled to traverse, seem less opaque than the central portions. A very thin shell of protoplasm at the surface is entirely destitute of granulations, though the yolk is certainly not provided with a distinct membrane, the so-called *membrana vitellina.* Toward the centre of the sphere the opacity is not, however, a constantly increasing one, for at or near this point there appears an elongated lighter portion, which is not distinctly limited, but shades gradually into the darker surrounding portions. This is caused by the absence of yolk granules from the central part of the vitellus. I have not been fortunate enough to secure an egg in which this central spot was perfectly spherical, as did Warneck; already a lengthening had taken place, and in most cases it appeared as two contiguous luminous areas. These become more extensive in the course of a few minutes, and soon appear so displaced that one is much nearer the surface of the yolk than at first. It is only in exceptional cases that these light spots can be seen, previous to the time when one of them appears near the surface. The more superficial spot is then the more conspicuous. As these draw nearer to one side of the yolk, the granules seem gradually to recede from that side toward which the clear bodies are tending, so that a considerable portion of the yolk appears comparatively transparent. After several minutes the outer spot reaches the surface, and is less sharply marked, probably because of the increasing transparency in the surrounding substance. The deeper spot is now very near the centre of the yolk, and only faintly indicated. After a short time the outer spot is flattened against the surface, and gradually acquires a greater superficial extent. There is now a slow accumulation of perfectly clear protoplasm at this side of the yolk; it is thickest where the light spot first touched the surface, and thins away gradually on all sides. This is all accomplished in about an hour after extrusion, though liable to some variation, the changes being more rapid in proportion to the elevation of temperature.

Up to this time the egg has remained without perceptible change of outline. With continued increase in the extent of the cap of clear protoplasm, which in section appears crescent-shaped, there is a slight elongation of one axis of the yolk, which gradually becomes more noticeable in the form of a low conical elevation at the side already indicated as that toward which the central spots tend. For the sake of precision I will call this the *animal* pole of the yolk, the opposite, the *vegetative* pole.

Thus far the changes, whether within or without, have been so slow as to be recognizable only after the lapse of some minutes; but now there begins at the middle of this crescent-like thickening a more rapid movement. The centre of this clear portion of protoplasm rises promptly in the form of a low, rounded eminence, of limited extent, which first becomes somewhat conical, and then assumes a more rectangular outline, in that its sides become nearly parallel. In this condition, it is really a low cylinder, with one end free and rounded, the other in continuation with the vitellus. (Fig. 1.) Sometimes this elevation seems to remain almost entirely free from opaque substance; at other times, granules accumulate to such an extent as to make the central portion of the protuberance appear very dark in transmitted light, and correspondingly white when seen by reflected light. The outline of the protuberance is sometimes slightly irregular and angular, although usually it is quite full and rounded. Without cessation it continues to change, principally by the mutual approximation of the sides of the cylinder at its base. This approximation takes the form of a constriction which is at first (Fig. 2) a broad furrow extending all around the cylinder. This furrow gradually becomes narrower and deeper (Fig. 3), and the excrescence which is thus being cut off takes a distinctly rounded form. The granulations now often appear gathered into the distal portion of the protuberance. Finally, the constriction deepens until there is only a slender thread of protoplasm joining the smaller and the greater sphere. This often persists for some time (Fig. 15), but finally ruptures, and sets free a small spheroidal body, with perfectly sharp and delicate outline, which is the first "polar globule." It is only five or ten minutes from its first appearance till it has the form of a sphere attached by a slender thread.

During the formation of this first polar globule other changes are taking place, to the consideration of which it is now necessary to return. The elongation of the yolk in the direction of the animal radius (as I shall call that radius which terminates in the animal pole) is very soon followed by its flattening in the direction of the same line. It is the

animal rather than the vegetative pole which shows the greater degree of flattening. This modification of the general form of the yolk reaches its maximum as the constriction at the base of the polar cylinder begins to deepen. But it is not alone a flattening which is noticeable at this time: the whole contour of the yolk becomes conspicuously modified. Whereas, at the first appearance of the protuberance, it has already become slightly flattened, it still remains symmetrical as regards the polar axis. Very soon, however, it becomes irregular, and more or less angular in its outline, and often appears remarkably unsymmetrical. During these few minutes it is constantly undergoing a slow change of form, which seems to affect every part of the yolk, and to be accompanied by redistributions of the granular substance of the vitellus, so that now one and then another portion becomes more opaque. As the detachment of the polar globule comes nearer to realization, these changes become less noticeable,* and finally, when the act is completed, the yolk has resumed its spherical form, and shows the same clear, even outline which had previously characterized it for so long a time. At the close of this act, a single, poorly defined clear spot is seen near the surface at the animal pole. The region of this pole still retains to a considerable extent its transparency, and a thin surface portion of clear protoplasm envelops the yolk on all sides. It is thickest at the animal pole, and thinnest at the vegetative. The portion immediately underlying the polar globule sometimes presents a peculiar striate aspect, which I have been unable fully to explain by other methods of study. The appearance is that of fine parallel striations, sometimes having the same direction as the animal axis (Fig. 49), sometimes oblique to it, or, on the other hand (Fig. 27), of two systems of parallel lines crossing each other at a considerable angle. These systems of striations seemed to be changing in position, yet without any recognizable regularity. They are probably astral rays or fibres of a nuclear spindle. (See below.) Gradually the vitelline granulations encroach on this peripheral clear layer, and it almost or entirely disappears.

Returning now to a consideration of the smaller sphere, it is found that the first polar globules differ considerably in size (25 μ to 40 μ), in different eggs, even though the yolks be of uniform diameter. When entirely detached, the polar globule is quite spherical, and remains for a

* In another species of Limax I have seen, since the above was written, very prominent pseudopodal elevations of the yolk at the animal pole toward the close of the formation of the second polar globule (Fig. 95). Compare the explanation of the figure.

short time tangent to the vitellus; but soon there appears between the two a perceptible interspace, which continues to increase for some minutes. The polar globule occasionally becomes removed a distance equal to its own diameter; more frequently, it is somewhat nearer the vitellus when this separation ceases. It often remains for some time at a distance from the vitellus, and then the interval gradually diminishes again. At first I was inclined to think this might be due to a slow change in the form of the vitellus, and that the motion of the polar globule was consequently more apparent than real; but the more I have watched it, the less have I been able to satisfy myself that such is really the case.

About an hour after the appearance of the first polar globule, — during which the external form of the yolk has remained without noticeable change, the crescent-shaped accumulation of clear protoplasm has disappeared, and the clear spot has become obscured, and again more distinct, — there begins again the accumulation of clear protoplasm at the animal pole, which, as before, varies much in its extent in different eggs. (Figs. 5, 10.)

Two or three times I have noticed just at this epoch a very peculiar behavior on the part of the first polar globule (Figs. 11–13). It seemed suddenly to give way on the side directed toward the vitellus; and its substance, which became as suddenly changed from almost complete transparency to a granular and opaque condition, was rapidly projected toward the animal pole of the yolk, with which it seemed to come in contact. This certainly does not always occur, nor can I offer any satisfactory explanation of its occasionally happening at this particular instant, i. e. just before the first appearance of the second polar globule. I have thought it might be due to the possibility that a change in the form of the yolk causes the rupture of an unobserved delicate connecting filament of protoplasm. But that does not seem very probable, inasmuch as the distance between the vitellus and the globule is such as to allow the discovery of such a thread of connection, if one really exist.

Occasionally there is to be seen in the yolk at this time a second clear spot, which lies much nearer its centre than the one which has now come to the surface and has become partially lost in the crescent of clear protoplasm. Usually one sees nothing of this deeper spot, owing to the abundance and opacity of the vitelline granules.

The changes which accompany the production of the second polar globule are so nearly identical with those which mark the appearance of the first, that attention need be called to only a few points in which they seem to differ.

I have noticed that very often the constriction about the base of the second polar cylinder advanced much more rapidly from one side than from the other, so that the axis of the cylinder, or becoming globule, regularly assumed a direction quite oblique to that of the polar axis of the yolk, and that (Fig. 19) consequently the point of final attachment was uniformly at some distance from the animal pole.

The second globule is very often somewhat smaller than the first, having three quarters or only two thirds the diameter of the latter. When first detached the second seems to push before it the first, but at length they assume positions alongside each other in contact with the vitellus.

I have never observed the *formation* of more than two polar globules, and never, when traced under normal conditions, a less number. Neither have I seen anything which could be compared to a division of either of the already formed polar globules, nor yet, with the exception of a single somewhat doubtful case, any instance in which as many as *three* globules existed near a single vitellus.

I have noticed no other constant differences in the polar globules. The interval between them and the vitellus increases after the detachment of the second globule, but subsequently both come to lie much closer to the yolk, almost always in immediate contact with it. Both often retain a spherical form for a considerable time, during several successive segmentations at least. In other cases, one or both exhibit an irregular and wrinkled appearance, due most likely, in all cases, to such a collapse and partial loss of substance as have in several instances been directly observed. I believe that there is no regularity about this, and that one globule is quite as likely to present this appearance as the other, if only one is thus affected. In this collapsed condition they continue to exist even in the most advanced stages. As is well known, they take no part in the formation of the tissues of the embryo.

We will now return to a consideration of the vitellus. Much as in the case of the first polar globule, the changes in the form of the yolk which accompany the production of the second are followed by an externally quiescent state, in which the vitellus, having once more assumed the spherical form, *seems* to be resting from its labors. The crescent-like shell of clear protoplasm at the animal pole again suffers the vitelline granules to encroach upon its acquired territory, so that the observer sees only a very neatly outlined sphere. It appears whitish, or slightly yellowish, in reflected light, and more or less opaque when viewed with transmitted light; it hangs suspended in the albu-

men like a planet, and at its side the two polar globules, of the same exquisite outline, are poised like a pair of satellites. A careful examination, even with reflected light, shows that the halves of the sphere are not quite alike. Notwithstanding the disappearance of the crescent as a distinct feature, the animal half still appears more glassy and not so white as the vegetative hemisphere, yet the one passes into the other without any abrupt transition. In transmitted light the distinction is even more apparent. Near this more translucent animal pole one may sometimes see a faint circular spot (Fig. 6) of still greater clearness, fading away gradually on all sides, but more often only a general translucency of this half of the yolk is noticeable. Even this gradually vanishes, and there follows a period of still greater obscurity. The granulations are grouped in ill-defined shadowy masses, which one feels like comparing to clouds, and again are re-grouped and re-distributed, apparently without definite order or effect. Sometimes, however, these changes have been observed to have considerable regularity. The most common appearance, though this has been seen only a few times, is that of a nearly equatorial zone (Figs. 21, 33, 36, 51) of protoplasm, from which the vitelline granules are almost wholly eliminated. The position of this zone varies somewhat in different eggs, and in the same egg has been observed gradually to alter in form and extent. Soon after its appearance, it is seen as a band of narrow surface exposure, but of great depth, so that in optical section (Fig. 33) it extends to near the centre of the vitellus, becoming thinner the nearer it approaches this point.

The outline thus presented in a section is that of two narrow wedges with their bases lying in the surface of the yolk near its equator, and their apices directed toward, and almost reaching, each other. In the course of twenty or thirty minutes the zone has become broader (Fig. 36), and a sectional view shows that the deeper edge, corresponding to the apex of the wedge, has become much rounded, so that the zone is now limited to the more superficial portion of the yolk. After twenty minutes more have elapsed, it has become still further restricted in its centripetal extension (Fig. 34), and is rapidly becoming indistinct on account of the encroachment of yolk granules. But these changes, as well as the less regular fleecy appearances which are more frequently observable, are accompanied by no corresponding alterations in the contour of the vitellus as a whole: the latter stills retains its simplicity of form.

Such changes as have just been traced are not the only ones going on within the yolk at this time, although it is only in favorable cases that one has a view of other possibly more important phenomena. The

region of the animal pole, never losing wholly its unlikeness to the rest of the yolk, after a time shows a faint light spot of more or less circular form. At first this spot is always poorly defined, and in many cases remains thus as long as it continues to be visible. In other cases, especially when it is nearer the animal pole, one may at length discover a clear-cut delicate outline, which always remains concave toward the centre of the spot. The latter is usually circular, but sometimes it is oval, and sometimes it has the form of an irregular body with rounded angles. This nuclear body continues gradually to increase in size, and at the same time to undergo *slow changes of form*, which, however, have never been seen to exceed the limits above indicated. When it has reached the size of the smaller polar globule, or somewhat earlier, a second like clear spot is seen lying deeper in the vitellus, and consequently less clearly defined (Figs. 21, 36). These bodies at first appear homogeneous, and less refractive than the surrounding protoplasm. Very soon, however, a few (1 – 3) small highly refractive corpuscles (nucleoli) may be seen in them at some distance from each other. They change their relative positions only slightly, as though passively shifted by the changes in the form of the nuclear body. The corpuscles increase in number, but I have not observed a division in any of them. The increase in the size of the nuclear bodies is quite gradual; they may attain, however, (Fig. 65,) a very considerable diameter (35 μ). They are respectively, the first, the so-called *egg-nucleus*, or *female pronucleus;* the second, the *male pronucleus.*

The formation and growth of the female pronucleus, which occupies from one to two hours, according to temperature, constitutes the last series of changes which belong to this head, — the phenomena of maturation, — and we may now direct our attention to the results obtained in studying this phase of egg development by other means.

For the purpose of pursuing the phenomena transpiring within the yolk, — which for the most part can only be traced with difficulty, or not at all, in the living specimen, — one may have recourse to treatment with various reagents. Acetic acid has furnished the means to this end in the greater part of my studies.

The condition presented by the least advanced eggs which I have been fortunate enough to secure was such as to contribute almost nothing to the solution of the question, What is the exact relation between the germinative vesicle and the first, or maturation, spindle? The eggs of Limax are not favorable objects for the study of this important question, — which has of late been agitated with such a fair prospect of a

satisfactory solution, — and for this reason: the changes accompanying the metamorphosis are certainly initiated, and probably almost always wellnigh concluded before the egg is laid.

At any rate, I am sure that certain of the eggs (Fig. 39) I have treated were taken immediately after deposition. They were immersed in a weak preparation of acetic acid; and yet, as before remarked, they were so far advanced as to afford little or no evidence toward the solution of this question. The yolk thus early subjected for several hours to the action of weak acetic acid within the normal egg envelopes, and then carefully freed from all enveloping substances and treated with Beale's carmine, shows already two well-marked stars, whose peripheries are in contact, the whole forming the figure recently named by Whitman "archiamphiaster," to distinguish it from similar figures, known as "amphiasters," which arise later in the history of the egg.

This archiamphiaster occupies the middle of the vitelline sphere. The two stellate figures composing it are of equal size. Each is formed by straight radiating filaments of protoplasm, which converge from all directions toward an imaginary centre, which they never appear to reach.

These filaments are differentiated portions of an otherwise nearly homogeneous protoplasmic mass, which has a spherical form, and tolerably definite, though by no means sharply marked outline. The extent of these two spheres is marked by the encroachment of the coarse deutoplasmic granulations held in suspension by the remaining protoplasm of the yolk, and their outlines are consequently more or less definite, as this encroachment is more or less abrupt. In no case is this so sudden as totally to obscure the continuation of a few of the radiating lines, for a short distance, into the more granular portion of the protoplasm.

As at their peripheral ends, so, too, at their central extremities, these filaments do not all terminate at a uniform distance from the mathematical centre of their respective spheres. Consequently there is a small space immediately surrounding this imaginary point of convergence, which, although in general of a spheroidal form, may be much less regular in outline than a circle, and is not very definitely circumscribed. In optical section it appears as a more or less circular homogeneous "area." It is only a little more deeply stained in carmine than is the surrounding protoplasm.

While most of the filaments composing the stars, or suns, are of the same thickness, there seem to be a few that are rather more prominent. These latter occur at intervals of 30° to 60° throughout the suns. It is

not always the stouter lines, however, which are traceable farthest from the centre.

The diameter of each of these sun-like or astral spheres is about one third that of the whole vitellus, and their centres are so situated as to divide the diameter of the yolk into three nearly equal portions, so that the two astral spheres appear at first sight to be simply tangent to each other. A more careful examination shows that this region of contact presents important, though not prominent, modifications of structure. All the fibres for some distance around the point of tangency are *continuous* from one sphere to the other. The stout fibres are here comparatively more numerous than in the other portions of the figure, and all are more or less *curved;* those farthest from the central point of contact are most curved. This bipolar mass constitutes a spindle-shaped body, having its apices at the centres of the two spheres. It is the "Richtungsspindel" of German writers. I shall call it the "maturation spindle." The fibres which help to form it differ so little from the radial filaments of the suns that it is difficult at first to distinguish between the two. There is, however, still a third peculiarity which helps to emphasize this difference. The fibres of the spindle are slightly thickened midway between the two poles. These thickenings form the nuclear plate (*Kernplatte*) of Strasburger. From the results of recent observations made on more favorable objects by numerous European observers, there is every reason to believe that this spindle-shaped body is the result of the direct metamorphosis of the germinative vesicle, or at least of a part of it. A careful examination, however, of all the early stages I have seen, has failed to show satisfactorily anything of the germinative vesicle, which a somewhat earlier stage would probably have disclosed.* I cannot avoid repeating the fact that, at this stage, the spindle fibres are very inconspicuously differentiated from the radial filaments. Except for much more satisfactory views at a later stage, I confess I should have been somewhat sceptical about the existence of such a structure distinct from the stellate figures.

It will be noticed that already the vitellus has no longer an homaxial form, but is monaxial. The differentiated axis corresponds to that of the maturation spindle; and its two poles are, so far as I have been able to discover, absolutely alike (haplopolar condition). This state

* In one case (Fig. 39) I saw near the plane of the nuclear plate outside the spindle a few irregularly shaped bodies considerably larger than the granulations surrounding them, which may possibly have been remnants of the germinative dot, or of the membrane of the germinative vesicle. If not the remains of nuclear substance, I know of nothing with which they might be compared.

is of short duration. At a subsequent stage it will be seen that this monaxial *haplo*polar form is followed by a monaxial *diplo*polar. Potentially, I believe, this diplopolar condition already exists. In the present state of our ignorance as to the nature and residence of the forces which control the phenomena connected with maturation, it is not possible to find direct evidence of such a state at this early stage. In the eggs of many other animals, the diplopolar condition is manifest much earlier.*

The protoplasm of the yolk not embraced by the spindle and amphiaster is closely crowded with highly refractive deutoplasmic granules, which do not exhibit any system of arrangement, save that they are uniformly distributed. These granules are irregular in form after the action of acid, and they vary considerably in size. In any optical section, the peripheral portions of the yolk appear more translucent than the central portions, not necessarily because of less abundance or less density of the deutoplasmic elements, but simply from the diminished amount of yolk substance which the light has to traverse to reach the observer's eye.

It would not be true, however, to say that the granulations extend quite up to the surface of the egg. There is a very thin, almost imperceptible layer of substance, free from deutoplasmic elements, which forms the outer envelope of the yolk. This is in no way to be considered as a vitelline membrane; however sharp the external boundary may be, the internal portion merges so gradually into the yolk substance as to afford not the slightest ground for assuming that it is a distinct membrane. It is hardly necessary to add, that there is no evidence of a double contour, and that all attempts to separate as a distinct structure this outer condensed portion of the yolk are quite futile.

As will be seen from Fig. 39, the uniformity in the distribution of the yolk granules is interrupted at irregular intervals (a). Spaces immediately contiguous to the surface of the yolk appear to be quite destitute of granules, and the corresponding portions of the surface are often raised into transparent, boss-like protuberances. These spaces — a dozen or more in number — are irregularly distributed over the whole yolk. They at once become conspicuous when the egg is placed in

* I am unable to say whether the axis of the yolk corresponding with the axis of the maturation spindle is identical with that which becomes differentiated at the formation of the polar globules. If so, then the migration of the spindle is only a motion of translation along this axis; but, on the other hand, if the spindle at any time assumes a position oblique to the radius which passes through its centre, it is probable that the axes are not the same.

Beale's carmine. One may perhaps seek an explanation of this appearance in a want of uniformity in the imbibition which takes place as soon as the egg comes in contact with the ammoniacal carmine. This view is, moreover, strengthened by the appearance of the exposed, thickened layer of protoplasm which envelops the yolk. For in the region of these spots the outline becomes less dark and conspicuous, as though a softening of the envelope had here allowed a portion of the protoplasm to become less dense, and therefore feebly refractive. On the other hand, a subsequent falling in of the surface at these points — such as might naturally be expected, from the above explanation, to follow when the object is placed in a more dense fluid, like glycerine — has not been observed; on the contrary, these areas continue to be raised conspicuously above the common level of the surface of the yolk. The protuberances have the appearance of quite naked, protruding portions of clear protoplasm. Whether they are anything more than the result of artificial ruptures of the cortical substance of the vitellus it is difficult to say. That they may be due to the presence of spermatozoa, which have already penetrated the yolk, is perhaps not impossible; but it appears to me unlikely from the irregularity in the size and configuration of the spaces, as well as from the entire absence of any regular arrangement (stellate) of the granules in the surrounding protoplasm. I have not discovered any specially modified central portion, nor any difference between the spots and the immediately surrounding protoplasm in the facility with which staining is effected. I have not observed anything of this kind in eggs of more advanced stages.

What has been said of this earliest stage is based, unfortunately, on a limited number of specimens; and, although my notes are unequivocal in declaring the eggs in question to be fresh-laid, I have not been able to entirely free my mind from the suspicion that, after all, they are much older than I have given them credit for being. The very considerable size of the amphiaster is perhaps in itself enough to suggest the possibility that they were nearly ready to effect the first cleavage, and that the aster is, consequently, the first cleavage amphiaster.

The considerations, however, which incline me to the belief indicated above are, in addition to the notes proving the freshness of the eggs, the following: —

1. *The almost exactly central position of the amphiaster.* This, it is true, would hardly be a safe criterion to distinguish this amphiaster from that of a second *archi*amphiaster, (as we shall presently see,) but is, it seems to me, very strong evidence that we have not to do in this case

with the amphiaster of the first cleavage nucleus; for the latter has a conspicuously *eccentric* position, — it lies nearer the animal than the vegetative pole.

2. The somewhat sharper limitations of the protoplasmic spheres (stellate figures) than prevails at the time of the first and subsequent segmentations. And, finally, —

3. The appearance presented by the thickened envelope of the vitellus, which seems to possess less consistency than during the later stages, and even at intervals to be altogether interrupted. This I take to be an indication of want of age.

I have not been fortunate enough to secure eggs in which the maturation spindle exhibits a position oblique to the radius passing through its centre, if I except a single specimen (Fig. 53), which I am inclined to consider as a case presenting the second rather than the first archiamphiaster.* (See pp. 206, 207.)

The next stage which has been observed (Fig. 45) is one in which the first maturation spindle has a radial position, one of the stars being at or near the centre, and the other reaching with its rays very near to the surface of the vitellus. The peripheral extremity of the radius in which this first spindle lies marks an important place in the topography of the egg. From this time on it forms a cardinal point, to which one may refer all changes of form. It is the point which we have already desig-

* The reasons for considering Fig. 53 as that of the *second* archiamphiaster, instead of the first, are: —

1. The egg is one of a series of seven taken immediately after their deposition by the slug. Four of these were at once subjected to the acid, and all show the first polar globule in an advanced state of formation. This was submitted to acid nearly thirty minutes later, and consequently would have had time to accomplish the elimination of the first polar globule, and effect the metamorphosis which its supposed condition implies, provided it was equally advanced with the others.

2. The appearance of the yolk at the surface near the more superficial of the two asters. In the shallow depression of its surface and the ragged edges which it presents, the yolk so completely resembles the appearance of the vitellus after the production of a polar globule, that I am inclined to believe that the globule has been detached and escaped notice.

3. The smaller size of the peripheral aster. It is probable that the internal star of the second archiamphiaster is the more direct (if not the exclusive) genetic successor of the single star remaining at the closing phases of the detachment of the first polar globule, and therefore may be expected to be somewhat more conspicuous than its more recently formed companion.

This last argument would have no weight if the second amphiaster arises, like the first, from the metamorphosis of a typical nucleus, since then both stars would be of equal age. (Comp. p. 206.)

nated as the *animal* pole. It is often spoken of as the *formative* pole, in distinction from the opposite and often less active *nutritive* pole.

In this condition, then, the egg manifests a diplopolar state, which may — doubtless must — have had a potential existence before; a state which is now most emphatically expressed by its internal structure, and which seems never to forsake it altogether. Whether, however, this monaxial state be identical with that which one observes in the earlier condition of the egg (Fig. 39) must be left undetermined for the present.

In this stage the *maturation figure* (i. e. the whole internal figure) presents itself as a compound structure, composed of the *spindle* and two nearly spherical masses of protoplasm, having the ends of the spindle as centres, and traversed by fibres radiating from them, — in short, the two asters of the so-called archiamphiaster.

The length of the *spindle* is a little more than one third the diameter of the vitellus, and its greatest thickness is somewhat less than half its length. Its outline, when viewed *en face*, is evenly curved, tapering from its greatest thickness at the equator toward either pole, where it is lost in the rays near the centre of the aster. The spindle embraces a large number of fibres, — probably not less than thirty or forty, — continuous from pole to pole. These fibres are considerably more conspicuous than the radiating lines of the asters. The intervening substance of the spindle appears structureless, and much less refractive than the substance of the fibres. Some of the latter exhibit, in the equatorial plane of the spindle, thickenings of considerable size.

It does not seem that all the fibres present such thickenings; nor, on the other hand, am I quite certain but that some of these apparent thickenings are really unconnected with any fibres, — at least their irregular distribution has suggested the possibility of their being detached, without having afforded as yet a sufficiently satisfactory demonstration of the existence of such independent granules. A view along the axis of the spindle (Fig. 46), while it affords pretty satisfactory evidence as to the relative position of the thickenings in the equatorial plane, does not prove sufficient to settle the question, for the reason that the fibres are so minute as to be almost entirely obscured by the overlying star. In the view thus had, one finds the number of thickenings to be about twenty, and that they are not distributed with any very clearly defined order. In a later stage, however, we shall find that the number is considerably greater, and that they are more conspicuously subject to a definite plan of arrangement.

The two stars, or, more exactly, spheres, of the first maturation figure already present a difference which is not diminished as the development of the egg advances. Not alone that there is a noticeable difference in the magnitude of the two stars, constantly somewhat to the disadvantage of the peripheral one, but, further, there is a sharpness to the limitation of the outer star which is missed in the outline of the central figure. Nor is this attributable solely to the obscuration produced by the rays of light from the deeper star having to traverse a greater mass of granular vitelline substance than do those from the more superficial figure. No doubt that fact enhances the difference, but the primary cause seems to lie in the more complete exclusion of the granular elements of the vitellus from the superficial than from the deeper sphere. In another point, too, these two stars may differ: the rays of the outer star, instead of presenting that rectilinear appearance which characterizes those of the deeper star, appear uniformly curved in a like direction (Fig. 47) when the star is viewed from the animal pole. This spiral arrangement of the radiating fibres of one or the other of the amphiastral stars is a phenomenon of not uncommon occurrence, and will demand a more extended consideration a little further on. It is a less constant feature of the outer star than the peculiarities previously described. Aside from these differences, there seems to be no noticeable distinction between the two asters or their relations to the portions of the spindle so closely connected with each. There is, however, one feature which very soon makes its appearance, or may perhaps be already detected in a faint degree, — I mean a modification of the form of the superficial sphere, which is accomplished at the expense of the diameter which coincides with the polar diameter of the yolk. To be more exact, however, this flattening in the direction of the animal radius is most conspicuous, if not exclusively apparent, on the outer hemisphere of the superficial aster. It is a modification which increases with the motion of translation which the whole internal figure is destined to undergo along this animal radius; it is perhaps only one of the physical effects resulting from the motion.

When this maturation figure is spoken of as though it were something by itself, it is not to be understood that it is a sharply defined object with definite boundaries separating it from the remaining portions of the yolk, but, on the contrary, it should be distinctly stated that at this stage the transition from the maturation figure, on the one hand, to the enveloping portions of the vitellus, on the other, is quite gradual.

The latter is characterized by the deutoplasmic elements, which are

evenly distributed through its mass, save where they come within the reach of the influence which determines the differentiation of the substance of the maturation figure into less refractive and more refractive fibrous portions having a definite arrangement. Here these coarse deutoplasmic elements are made to assume a corresponding radial relation. They decrease in abundance toward the centres of the stellate figures.

The figure of the whole vitellus is that of a sphere, which may already be slightly flattened in the direction of the only differentiated axis.* The portions which are most deeply stained in carmine are, first, the equatorial thickenings of the spindle, and after them the central portions of the two asters. The gradual increase from periphery to centre in the density of each aster makes it more and more difficult, as one approaches the centre of the star, to distinguish the compact protoplasmic rays from the protoplasm in which they are imbedded. For this reason the "area" is not always at this time a region definitely circumscribed by the central terminations of the radial fibres.

In the stage just reviewed we have seen the first archiamphiaster fully formed, and already advanced to a position such that a continuation in its motion of translation will necessarily make itself at once apparent in the general outline of the yolk. Such a modification is, in fact, the thing which, in the next stage (Figs. 43, 48), most forcibly attracts attention, — not, however, in the manner one might have anticipated. The whole vitellus becomes conspicuously flattened in the direction of its polar diameter, and at the same time presents at the animal pole, as a sort of compensating change, a slight elevation. The latter becomes prominent in proportion as the vitellus, as a whole, undergoes further depression at this pole of the egg. It is as though the vitellus at the animal pole were to sink gradually away, leaving the peripheral end of the archiamphiaster protruding beyond the general outline. The latter, as seen from either pole of the main axis, remains that of a circle, or at least presents only very slight and inconstant deviations from that form; but the outline, as seen in *profile*, becomes altered, not simply, as it would seem, by the protrusion of the maturation figure, but by a concomitant flattening of the adjacent portions of the vitellus. The relation of these two acts to each other and to their cause will be considered hereafter. The resulting outline is like that produced by the insertion of the arc of a smaller circle into that of a greater one. The

* For the peculiar appearance of the outline at the animal pole of the vitellus in Fig. 45, see the explanation of the figures.

inserted arc corresponds to a circle having approximately the diameter of the outer stellate sphere. The resistance offered by the yolk envelope is, however, so considerable as to cause a decided flattening of the external half of the peripheral stellar sphere. It also results from this resistance that the outer ends of the radiate filaments of the outer star are curved away from the polar axis, and finally bent backward, — much as the hairs of a soft brush would be when gently forced against the concave surface of a highly curved watch-crystal. The effect of this curvature in the filaments is at first quite deceptive; for it unmistakably suggests to the observer that from the apex of the protuberance there is a funnel-shaped depression extending to near the centre of the radiate figure, — a depression in form something like that of the corolla of a morning-glory. This illusion is further heightened in specimens which have been stained, by the fact that this apparently invaginated portion is only slightly tinged in comparison with other portions of the stellate figure, or the yolk (Fig. 43). It is, however, very certain that no such depression actually exists, as one may be convinced by carefully rotating the egg and focusing the instrument so that the centres of both stellate figures are seen distinctly at the same time. The axis of the spindle then lies in a plane parallel with that of the microscope stage, and its whole length is exactly in focus. If there were such a depression at the surface which lies in the continuation of the spindle axis, it should be observable in the outline of the yolk; but, on the contrary, the very transparent portion of the peripheral star shows a sharply defined outline, convex externally, and continuous at each end with the outline of the rest of the yolk (Figs. 43, 48).

When the egg has been subjected to the action of acetic acid, the very fulness of the outline at the animal pole, as contrasted with the more or less shrivelled and irregular outline of the rest of the vitellus, is to me an indication of the high state of mechanical tension to which this portion of the surface is subjected.

At this time the superficial portion of the protuberance seems to become differentiated into a thin membrane with double contour, which is continuous at the margin of the elevation with the less conspicuously differentiated outer layer of the rest of the vitellus. This membrane is at first of uniform thickness over the whole surface of the protuberance. It is only in a subsequent stage that it assumes a different and peculiar appearance.

As already indicated, the radiating fibres of the peripheral aster suffer a bending back, which changes their original direction more or less,

according as they would naturally lie near to, or more remote from, the continuation of the spindle axis. The course of some of the fibres of even the deeper half of this star is thus affected. The result is a gradual diminution in the number of fibres in the outer half of the aster, and their greater concentration near the equator of the astral sphere. It is this increase in the number of the fibres near the equator of the aster, together with their arched course, which causes the peculiar funnel-shaped phenomenon already described. Such simple, and yet unique, modifications of the star are not the only ones to be found. While I believe the backward deflection of the fibres is an invariable, a mechanically *necessary* feature of this stage of the egg phenomena, I am not able to say as much of some other modifications; in fact, I am almost certain that the latter are to be found only occasionally. They may not, perhaps, on that account, prove less interesting. I refer to the very peculiar appearance sometimes presented by the outer aster, when one looks directly down upon the animal pole of the egg. Instead of seeing the fibres radiate in straight lines, as one might naturally expect and would find in the majority of cases, it will often be discovered that they are uniformly bent into a spiral, presenting thus a figure not unlike that of a turbine water-wheel (Fig. 56). The curvature in the cases I have seen (remembering that the observer is looking upon the animal pole) is such as would be produced by the peripheral ends of the fibres being moved in the direction in which the hands of a clock advance, while the centre remained fixed, or by an opposite rotating motion of the axis of the spindle when the peripheral ends were immovable.

This spiral phenomenon has been observed even before the aster had caused any elevation of the surface (Fig. 47), but not before it had reached the periphery of the yolk. No phenomena corresponding either to flattening, backward deflection, or spiral arrangement of the fibres, have been observed in the deeper star up to this stage.

During the stage now under consideration the centre of the *peripheral* sphere becomes conspicuously modified. It is at length occupied by a circular, highly refractive homogeneous body, flattened in the direction of the axis of the spindle so that it appears oval in a profile view. This body, at times irregular in outline, appears to be surrounded by a clear zone of uniform thickness. The appearance may be due solely to reflection from the body itself. (Comp. Flemming, **'78**[b], p. 310.*)

* The numbers immediately following an author's name serve the double purpose of referring the reader to the list (p. 591) where the titles of papers are given, and of informing him at once of the approximate date of the paper in question.

The uniform thickness of the zone would favor this interpretation; on the other hand, it is sometimes too broad readily to admit that explanation.

This body is the centre of the peripheral radiation, and corresponds consequently to the centre of the deeper stellate figure. In the latter, however, I have not usually succeeded in finding any corresponding well-defined structure. As in the earlier stages, one sees only an irregular area, often homogeneous, but at times apparently made up of a small number of coalescing, not highly refractive globules. (Comp. Fig. 55.) In only one case (Fig. 48) have I seen anything like a sharply limited body in the centre of the deeper aster. In this case it was of about the same size as the peripheral body, and like it appeared slightly flattened in the direction of the spindle axis. Still, the outline was less sharp, and the flattening less conspicuous.

The impossibility of fixing with accuracy the absolute, or even relative, degree of advancement of different eggs at this period, deprives the observations in great measure of the value they might otherwise have. This would be especially perplexing were it not that one is at liberty, when the evidence is so uniform, as it fortunately is in this case, to use the observations of others, even though made on different animals. Not that there is no direct evidence of which to make use, but simply that it is less complete than it would have been, had the eggs been more transparent.

The *spindle* at this stage is not always prominent. In some cases (Fig. 43) it is with great difficulty that its fibres can be distinguished from the radiate filaments. At other times, when the superficial protuberance and the central body of the aster are already differentiated, its limits are very well marked.

Thickenings in its fibres are often not easily made out. Even where there is a prominent elevation of the yolk and a sharply marked body in the outer aster, the central zone of the spindle sometimes appears as hardly more than a continuation of the granular protoplasm which, in optical section, seems wedged in between the surfaces of the stellate spheres (Fig. 43). On the other hand, eggs in which the elevation is less pronounced (Fig. 48) may present a clearly marked median zone of thickenings, or even two closely approximated zones. Figs. 43, 48, (and others not reproduced on the plates,) evidently exhibit slightly different stages, although the eggs were deposited in one bunch, certainly not more than a few seconds apart, and were subjected to the same treatment. The prominence of the stellar elevation, which might

at first appear to be a fair index to the advancement of the individual eggs, seems to be of secondary importance ; for in this series the one (Fig. 48) presenting the least elevation is the one which, to conclude from the appearance of a subsequent stage, as well as from analogy with observations on the eggs of other animals, is in reality the most advanced. The evidence is found in the fact that there are two zones of thickenings here, while in the others only one zone is discernible. It has been clearly shown, by direct observation of living cells of both animals and plants, that this double zone arises by the splitting into halves of the single median zone ; and that such is really the case here cannot be doubted, as we shall see when we come to the investigation of the next stage. Moreover, a careful examination of the figures shows that the centre of the outer star is nearer the surface (whither it was certainly tending) in the eggs which, for reasons just given, we must conclude are the more advanced.

The spindle, as in the preceding stage, is usually very stout, its thickness at the equator being nearly half its length, which still remains about one third the diameter of the vitellus.

The arrangement of the granulations in the vitellus differs but little from that which prevailed in the last stage. There still exists a uniform distribution of these elements save where the archiamphiaster has caused their more or less extensive disappearance. Corresponding to the changed position of the archiamphiaster, the area of distribution is somewhat modified ; but otherwise I can discover no alteration in eggs subjected to hardening processes.

The further transfer of the archiamphiaster toward the surface of the vitellus is accompanied with continued changes, which affect its shape more than the general form of the vitellus. Instead of pushing before it the thin covering formed at the animal pole, and emerging from the surface as a complete spherical aster, the exposed half of the outer star suffers a further and marked change of form, as well as reduction of size (Fig. 50). The centre of this stellate figure now lies close to the surface of the vitellus, a thing which could only occur by a displacement in the more exposed radiate fibres. What the nature of that displacement is, can be inferred from what was seen in the last stage, where a mechanical deflection was so apparent.

From the position of the centre of the outer star, it follows that the latter is now much less than a complete sphere ; it is even much less than a hemisphere. The radial extent of the portion which remains is, however, about the same as before, that is to say, the radiating

fibres are of nearly the same length. Consequently the inner limitation of this outer aster — the surface which abuts upon the granular vitellus — is, as before, of nearly circular outline; the free or exposed portion, on the other hand, has not quite the form of the arc of a circle, for the curvature is sharpest at a point directly over the centre of radiation. The course of the adjacent rays is nearly parallel with this outline. The whole figure of the outer star is thus changed from a sphere to a form more resembling a biconvex lens, with its more highly curved surface directed outward.

The compact body which in the earlier stages existed at the centre of radiation still persists, although its form is further modified by a continued flattening. It is in the profile view that this body is most conspicuous. It corresponds, I believe, with that which Robin (**'75**, p. 34) calls in Nephelis "un espace clair circulaire, superficiel," but which has been better seen and more clearly depicted by Whitman (**'78**[a], p. 18, and Figs. 62, 63, C. P.), in the case of Clepsine. I shall have occasion to recur to this subsequently.

The modifications which the external aster has undergone, caused in part at least by its relation to the outer envelope of the vitellus, do not find their counterpart in a like modification of the deeper aster. A certain amount of change may also be observed here. This aster has also approached the animal pole; it has moved to a position at some distance from the centre of the vitellus; there is also a slight change in the extent of the radiate influence of which it is the expression, for the rays which reach out into the vegetative hemisphere are somewhat longer than those belonging to the opposite half of the yolk. Its peripheral limitation continues to be less sharply marked than that of the outer star. The inner ends of the rays, on the other hand, terminate at a nearly uniform distance from the centre of radiation.

The spindle itself still retains the robust proportions characteristic of the earlier stages. Its length has not suffered appreciable change, and its outline is less modified at the equator than in the earlier stages, when the bending at this place often appeared quite abrupt. The interstellate fibres are more distinct than formerly, and the centres of the stars continue to be the points of convergence for the two extremities of the spindle fibres. The thickenings of the latter are more or less widely separated, and appear as two distinct and conspicuous zones at equal distances from the equator. When seen lengthwise of the spindle, the numerous (40 to 50) thickenings appear arranged — more distinctly than in the previous stage — in the form of a *ring*,

neither border of which is sharply marked. Between the two zones of thickenings are stretched delicate nearly parallel threads, which I shall designate as *interzonal filaments.*

The granular elements of the yolk are distributed with the same uniformity as before, and are only so far modified in their arrangement as might have been anticipated from the changes in form, position, and extent of the archiamphiaster. The vitellus has again assumed more nearly the circular outline, aside from the protuberance caused by the archiamphiaster. That portion of the profile where this conical protuberance joins the sphere presents a very slight reverse curvature. In hardened eggs the contour of the low cone is constantly distinguishable from that of the remaining vitellus by a fulness and evenness which are quite as noticeable as in the preceding stages. This is the more conspicuous in some specimens from a thickening in the envelope of the vitellus at this pole. In extreme cases (Fig. 50) the thickness of this structure (*vm ?*) may reach $3.5\,\mu$ at the pole itself, but it thins out rapidly, so that where the base of the conical elevation joins the vitelline sphere it is quite indistinguishable. It is sharply marked from the underlying stellate figure, and presents in glycerine a clear, even, though not prominent, external outline. Its substance is finely punctate, a thing which causes me to question the interpretation (vitelline membrane) which I was at first inclined to give this structure. In some cases, apparently in this stage of advancement, it is represented by only a thin homogeneous cortical layer such as is depicted in the preceding stage (Fig. 43).*

The changes following upon the conditions last described lead directly to the production of a small, more or less spherical body at the animal

* I am in no way prepared to insist upon the identity of these two structures. As regards the origin of that which is to be seen in Fig. 43, I have no hesitancy in referring it to a cortical portion of the yolk itself. The structure exhibited in Fig. 50 presents peculiarities not easily harmonized with a like explanation of its origin. Foremost is the fact of its low refracting power; secondly, its finely granular structure; and, finally, its very unequal thickness within narrow superficial limits. Whether it may not be a comparatively thin fluid exudation from the animal pole of the yolk coagulated by the acid, or whether it may not owe its origin to the albumen surrounding this region of the vitellus, are possibilities which I have not been able definitely to accept or reject. If in any way dependent on the albumen for its origin, it is difficult to understand what should induce it to take this very peculiar form, and why it has so sharp and even an exterior. I can recall nothing in the development of other animals with which it may possibly be compared, unless perhaps with the exudation from the eggs of Batrachia, described by Bambeke and O. Hertwig.

pole, — the so-called *polar globule.* The steps in its formation have already been followed in the living egg, in so far, at least, as concerns the successive phases of its changing outline.

The slight annular depression marking the limit of the external aster in the previous stage gradually deepens, and the transparent fibrous protoplasm of the outer star, together with a portion of the more granular protoplasm in its immediate vicinity, is pinched off by this deepening constriction.

The internal alterations accompanying this process (Figs. 40 – 42) are in a measure only the continuation of those noticed in the last stage. The maturation figure has migrated farther from its central position. The external star has undergone a more thorough metamorphosis than the deeper one. The spindle has suffered a constriction at its equator, but its length remains nearly unaltered.

What is left of the radial structure of the outer star is discoverable within the polar globule. A few faint lines, lying mostly near its surface, are all that can now be seen; even these are not uniformly observable. When present, they are often so closely applied to the surface of the polar globule as to leave the observer for a time in doubt if he has not before him a series of striations or foldings in the envelope of the globule. The slightly serrated outline of that portion of the globule which in Fig. 41 faces the vitellus, seems at first to strengthen the latter opinion; but a careful inspection seldom fails to show that the markings are not all parallel, — that different systems cross at a slight angle, which could hardly be expected of surface foldings. The greater part of the contents of the polar globule show not the least trace of such a structure, but are simply either quite transparent and homogeneous, or show a very fine punctate appearance, in which a few larger granules are occasionally found. The structure of the central portion will receive attention a little further on. The body which formed the centre of radiation is usually no longer distinguishable, its substance possibly having become disseminated in the globule. Occasionally, however, (comp. Fig. 63,) one discovers, attached to the free pole of the globule and projecting inward, a prominent thickening (*aa'*?) which I am inclined to consider as at least a portion of this refractive corpuscle which has by this time gained an intimate connection with the envelope of the globule.

The deeper star, having moved further from the centre of the yolk, as already indicated, is now tangent to the inner surface of the vitelline sphere. The amount of substance that is brought directly within the influence of this star is slightly increased, principally on the side opposite

the polar globule. It should be remarked, however, that the extent of this influence, as evinced in the radiate structure, is subject to considerable variations in different eggs at the same stage of development. While a homogeneous area at the centre of this star is marked off at times with great distinctness (Figs. 22, 25), in other cases the central portion is less prominent. It may continue to present the appearance previously noticed, as though originating from a few irregular and poorly defined masses of nearly homogeneous substance. One side of this central area is marked in a conspicuous manner, as will be seen presently, when the structure of the spindle is considered.

The latter has now become very much modified, and, by the advance of the constriction, is made to assume successively different forms, until at length it is like two spindles placed end to end rather than a single structure. What we may call the outer spindle, i. e. the outer half of the original body, which now occupies the polar globule, is less spindle-shaped than the inner half. I have never been fortunate enough to see its fibres converge beyond the zone of thickenings, after the constriction has made its appearance at the base of the polar globule. A slight convergence of the interzonal filaments toward a point on the distal surface of the globule is, however, often observable (Figs. 22, 63). After further constriction the region of thickenings appears more expanded, as in Fig. 25.

The internal half often preserves for some time a fusiform appearance, though the theoretical apex at the centre of the stellate figure has no visible connection with its fibres. It is only the trend of the latter which indicates this point as coincident with the centre of the star. The place of interruption in the fibres is dependent on the size of the central area.

Already at the beginning of the constriction the two lateral zones (or nuclear plates) had migrated, the one to near the border of the deeper "area," the other to a corresponding position relative to the refractive body of the outer star. During the constriction the thickenings of the former are found grouped together at the periphery of this "area," in such a way as to form a circular disk rather than a ring such as was observed in the earlier stages. The outer of these migrating zones, on the other hand, has not diminished in circumference, but has spread out, and still presents the annular rather than the disk-like arrangement. When seen from the animal pole the spindle thickenings in the polar globule (Fig. 42) consequently appear as an ill-defined ring.

That portion of the interzonal filaments which falls within the polar globule is gradually drawn into the thickenings; at least the filaments as such disappear. That portion which remains within the vitellus, after suffering a diminution in thickness, probably disappears altogether; so that shortly after the separation of the first polar globule there is found in the vitellus only a single stellate figure near the animal pole. In the polar globule, on the other hand, there is no radiate structure, — simply a group of prominent granules which are conspicuous from the readiness with which they are stained in carmine. The globule often gives evidence of being limited by a special membrane, which must have come from the envelope which we have traced in its origin as a covering to the rising cone of the animal pole. As that envelope was often thick at the apex and rapidly grew thinner toward the base of the cone, so we find that the corresponding structure is often much thickened at the distal extremity of the globule (Fig. 22), — the point corresponding to the apex of the cone. This thickened portion passes gradually into the envelope of the sides and proximal face, which seldom shows more than a single contour line. Before the complete detachment of the globule, there is formed (Fig. 63), in the pedicel which still establishes a connection with the yolk, at a point corresponding more or less closely with the equator of the spindle, another thickening, which may be the equivalent of the cell-plate (*Zellplatte*) of Strasburger. It is a disk of considerable thickness, which extends quite across the pedicel, and is highly refractive. Though not directly observed, it is reasonable to suppose that the final separation is along this disk, most likely by its division into two plates.

The events which immediately follow the formation of the first polar globule seem to me to have been less clearly treated by those who have engaged in the study of the phenomena than any other portion of these remarkable changes. Nor can I add much to their elucidation; for it is only after carefully comparing the results of my summer's work that I am inclined to believe that there remains just here something of a gap in the continuity of the best observations. It has been customary for the *second* archiamphiaster to receive only a hasty description. Its origin has often been quite neglected. Because the result in the case of both maturation spindles is the production of a polar globule, the phenomena in the second case seem to have been considered of only secondary value in the search for what is new. At the beginning of this second stage careful attention is demanded to answer the question, How does the second archiamphiaster arise?

Does the spindle completely disappear? Or does the vitelline half persist? And if it remains, does its outer end become the centre of a new force, acting on the surrounding protoplasm to induce a new peripheral star? Or, if it vanishes, does the single star develop antagonistic poles which move apart, each taking with it the half of the great star left in the vitellus?

So far as one can judge from the observations that have hitherto appeared, the most nearly complete second archiamphiaster yet seen is one having a spherical central aster joined by a spindle to a very *incomplete* peripheral aster, whose centre of radiation lies in the surface of the vitellus. The latter in its greatest extension is less than the half of a complete star. That there have been important omissions from the history of the second archiamphiaster will at once be inferred upon consulting Fig. 23. The first polar globule has already been formed, but still remains loosely attached to the vitellus, and further held in place by fragmentary portions of the surrounding albumen of the egg (a). The second archiamphiaster is completely formed. Its axis coincides almost exactly with the polar axis of the yolk. It lies *wholly* within the vitellus, being nowhere tangent to its surface. The nearest point of approach to the surface is immediately under the polar globule. The composition of this second archaic figure is deserving of close attention. The two stellate figures which make up the most of its substance are joined by a spindle which is not very distinctly outlined.

Perhaps the most noticeable feature of the whole figure is the unlikeness of the two stars; such a difference as we have already seen (Fig. 45) in the *first* archiamphiaster. The outline of the deeper sphere is by no means sharp, for prominent rays here and there extend into the coarsely granular protoplasm for some distance beyond the majority of the radiate fibres. In the case of the more superficial sphere, on the other hand, the rays terminate at such a uniform distance from its centre that the outline is quite even, and almost circular, in whatever position it be viewed. Otherwise the two asters are much alike: the rays are straight in both, though more uniformly distinct in the outer than in the deeper sphere. The centre of each is composed of a poorly defined, not quite homogeneous refractive substance, as in corresponding stages of the first archiamphiaster.

The fibres joining the centres and together constituting the spindle are, as usual, slightly curved, and they already present inconspicuous thickenings in the equatorial zone.

The granulations of the vitellus, although for the most part evenly

distributed, show about the animal pole irregularities of arrangement, which at first sight give one the impression that the outline of the outer stellate sphere is not even. This is most noticeable when the view is upon the animal pole, as in Fig. 24. However, the archiamphiaster, seen in profile, shows — whichever way the vitellus is rotated about the spindle axis — that this appearance is produced by aggregations of granules quite outside the stellate sphere, and that really the surface of the latter is not invaded by these granulations.

Inasmuch as the stage just described has not been seen by other observers, it will not appear superfluous to state briefly the evidence that it is the *second archiamphiaster*. The stages with which this might most easily be confounded are without doubt that of the formation of the *first* archiamphiaster, and that of the *first cleavage* amphiaster.

1. The egg in question was one of four of nearly the same degree of advancement (Figs. 25, 22, 23, 57). Three of these were subjected to acid at intervals of ten minutes, the first (Fig. 25) being immersed on the appearance of a conical protuberance ; the second (Fig. 22), though ten minutes later, seems to have been hardly more advanced than the first. This one (Fig. 23), the third, was observed to have a conical elevation ten minutes before its immersion, although the elevation may possibly have first appeared a few minutes earlier. It must have been then at the *least* ten minutes after its appearance, and most likely more, perhaps even fifteen or twenty minutes, when the conditions here preserved became fixed. This in itself would be enough to preclude the possibility of the first mistake, even if the first polar globule were no longer to be discovered in contact with the vitellus. There can be no doubt, then, that the first polar globule had already been formed, and that consequently the figure in question could not be the *first* archiamphiaster.

2. The second possibility may not at first appear so easy of refutation. The position of the axis of the spindle relative to the already formed polar globule, it is true, is little in harmony with the interpretation of the figure as the amphiaster of the first cleavage sphere, and is exactly what we might expect of a *second* maturation spindle. Nevertheless, it might be urged that possibly the polar globule is no longer located at that point of the vitelline surface where it originated, and that consequently the relation of the spindle axis to the globule is quite valueless in determining the nature of the spindle ; for in that case the polar globule here figured might be the *second*, and the stellate figures accordingly could only be interpreted as belonging to the first cleavage

amphiaster. Aside from the fact that the egg in a living condition was under observation at least some ten minutes immediately prior to its immersion, during which time one could hardly have failed to distinguish a fully formed first polar globule, had such actually existed, there are other and sufficient reasons for construing the observations differently. Not only that the comparatively large size of the polar globule points to its being the first, rather than the second, and that a slight prolongation from one side (p) is evidence that it had not yet wholly severed its connection with the vitellus, but it is especially the evidence within the vitellus itself that makes the above interpretation inadmissible.

This can be understood only by reference to what will appear more fully in speaking of the amphiaster of the first cleavage sphere; namely, that the two stars of the cleavage amphiaster lie in a plane which is perpendicuar to the animal radius at a point much nearer the animal than the vegetative pole, and that they are of almost identical appearance, though often deviating considerably from a spherical form. (Compare Fig. 82.)

None of these conditions are fulfilled by the figure under consideration. There is no evidence that any one of the lines perpendicular to the axis of the spindle at its middle * terminates at the animal pole of the vitellus; and even if such evidence existed, the plane, which is perpendicular to such line and also passes through both the asters, would not be perceptibly removed from the centre of the vitellus. Moreover, while the two stellar masses are almost spherical, — and therefore unlike that which we might expect in the amphiaster of the first cleavage sphere (compare Fig. 82), — they differ from each other in the sharpness of outline already noticed, and thereby again fail to conform to the requirements of the indicated interpretation. Other objections, drawn from a comparison of this figure with the amphiaster of the first cleavage sphere, might be adduced in answer to the possibility of this explanation; but enough has been said already to place beyond doubt its true nature; it is the amphiaster that immediately precedes the formation of the second polar globule.†

* It is at once apparent from the figure that no such perpendicular could be a radius of the vitellus, from the fact that one end of the spindle is much farther from its centre than is the other.

† The possibility that one of the stars might be due to fecundation — might be the so-called male aster — has not been overlooked. But the intimate union of the two stars by means of a spindle which has an equatorial zone of granulations would make this extremely improbable, even if the method in which the two pronuclei become joined were less accurately known than at present. See pp. 224–229.

It is an important question, How does the second archiamphiaster arise, and what relation does it bear to the first archiamphiaster?

Very few observers have given this question special attention, and those who have are not all positive in their opinions. According to most of the descriptions given, the vitelline "half-spindle," which remains after the formation of the first polar globule, simply undergoes an elongation caused by the gradual recession of the single remaining aster from the surface; the internal zone of fibre thickenings disappears by the distribution of its substance to form the lengthening fibres of the new spindle; and there arises a second stellate figure whose rays converge toward a point of the surface where the peripheral end of the spindle remains. Such an origin could not be directly compared with the formation of the first archiamphiaster, or subsequent amphiasters: it must be at best a greatly abbreviated process, if at all comparable with the ordinary method of amphiastral formation. In all other cases *both* centres of radiation arise as *new* differentiations in the protoplasm, and only make their appearance when the nuclear substance has assumed a definitely circumscribed form; in this case (according to the authors) only one of the centres of radiation has the least claim to be considered new, and the nuclear thickenings do not become fused into a definitely limited nucleus.

The case (Fig. 23) to which I have called attention presents some evidence that the second archiamphiaster is not formed in so direct a manner as has been supposed. There is no absolutely incontrovertible reason for denying that this *complete* amphiaster may have been formed much in the manner above indicated for the incomplete one. It would only be necessary to assume an extensive migration from the surface on the part of the spindle and its asters, instead of a movement on the part of the deep aster alone. There are, however, some objections to this view. The spindle has the appearance of being formed in the ordinary way, rather than that of having its fibres drawn out; it is not so sharply defined as I should expect a spindle to be, if resulting from a drawing-out process; it is much broader, and its peripheral fibres more abruptly bent, than would be the case in that event. The fact of its being totally enveloped in the yolk is in itself more easily reconcilable with its formation in a normal than in an abbreviated manner, since in the former case the centres of radiation arise at points within the vitellus, and thus is avoided the necessity of supposing that there is a centripetal migration of the spindle.

We have seen that at the completion of the first polar globule the

lateral zone of thickenings belonging to the vitellus had already reached the edge of the central "area." The vitelline half-spindle has been seen gradually to fade, but its complete disappearance I cannot affirm from direct observation. It seems to me not entirely impossible that its filaments are absorbed by the zone of thickenings, and that the latter is actually converted, as in the normal method, into a nuclear structure, in the vicinity of which two new stars (the second archiamphiaster) make their appearance. Both of these (the existence of a veritable nucleus, and the formation of *two* new stars) are only assumptions. I have no direct evidence that such a nuclear structure intervenes between the two archiamphiasters, nor that the two asters are both formed about new centres. There are only very slight indirect signs of such a condition, — indications that only warrant the suggestion of a possibility. I will not on that account withhold the observations.

There is some reason for believing that the view presented in Fig. 53 is that of the *second* archiamphiaster in process of a rotation which would eventually have brought its axis into coincidence with the animal radius of the vitellus.* If such be the case, it seems quite probable that this whole figure originated from a nuclear structure, in much the same manner as the first archiamphiaster is known to arise from the germinative vesicle, and that consequently this second spindle was not *directly* derived from the first spindle, and that possibly both of the stars are new productions. The reasons already indicated for thinking it is the second, are certainly only meagre evidence to fill the place of the more complete observations which are needed, but may possibly suffice to make probable what I have stated as my conviction, that the figure is that of the second, and not of the first, archiamphiaster.

Perhaps the most noticeable feature of this amphiaster is the inclination of its axis to the supposed animal radius. This specimen is especially interesting, as it is the only one in which I have succeeded in finding evidence of this obliquity. Such a peculiarity has often been noticed by other observers in the case of the *first* archiamphiaster. It will be seen from Figs. 53, 54, that the spindle is not *radial* in position, The two asters are not of equal extent, the deeper being the larger. Such a difference in sharpness of limitation as I have seen in other cases is not noticeable here, or at least it is much less marked than in many instances. In neither star does the influence produce rays reaching to the periphery; in other words, the figure is wholly immersed in the

* See page 189.

vitelline substance. This in itself might have been a serious argument against interpreting it as the second archiamphiaster, had I not already shown from Fig. 23 the possibility of such a state of affairs. The centres of the stellate figures do not exhibit distinctly outlined "areas," and the radiate fibres consequently become gradually lost in the central darker portion.

I have spoken of the spindle; this exists potentially rather than formally. The substance lying between the two asters is much clearer than the surrounding protoplasm, that is, it is destitute of vitelline granulations. Its outline, though not sharp and definite, is sufficiently distinct to show that it has an almost spherical figure, tapering a little at the poles. The fibres which help to make it conspicuous are not large, nor are they evenly distributed through its substance. A view along its axis (Fig. 54) shows that it is only the peripheral portion of the spindle which is thus differentiated, and, further, that it is not developed alike on all sides. While the fibres on one side extend a third of the way toward the axis, on the opposite side they form only a thin layer. No median or lateral zones of thickenings are observable.

The impression conveyed by these observations is that the spindle is not yet completed, that it is rather in process of formation, and that the differentiation, commencing at the surface, advances with varying rapidity toward the axis of the figure. If it were absolutely certain that this is the second archiamphiaster, there would be little ground for the belief that it was formed by a simple elongation of the half-spindle.*

In Fig. 55 the amphiastral figure has assumed a strictly radial position, and the peripheral star has already reached the surface of the vitellus, where it induces a prominent protuberance. The inequality of the two asters is more noticeable than in the formation of the first polar globule, a phenomenon which must be prevalent if the superficial asters and the resulting polar globules are proportional in size. What seems most peculiar in the outer star is the limited extent of the radial influence on the side toward the centre of the vitellus, a feature not seen in other figures at this stage.† The deeper star remains central in posi-

* There is hardly reason to suppose that the egg seen at Fig. 53 is so far advanced as to present the amphiaster of the first cleavage sphere. Neither the stage of development, as inferred from eggs of the same lot, nor the position of the figure nor the inequality of the stars, seem reconcilable to such an interpretation.

† As indicated by a previous reference to this figure, the external aster presents in a marked degree, when viewed from the animal pole, the peculiar spiral arrangement of its radiate fibres which was seen in an early stage of the formation of the first polar globule.

tion and of wide extent; its rays straight; its centre not quite homogeneous.

The spindle, especially, appears very different from the condition presented in the preceding stage. It is well defined, long, and embraces few thick fibres, which are arranged in a close, narrow bundle. The middle third of the fibres seems somewhat thicker than the terminal thirds, but no other indication of either equatorial or lateral zones can be made out. From the position of the centre of the outer radiate figure, which is still at some distance from the surface, it may be inferred that the stage here shown antedates the formation of the equatorial nuclear plate; however, the gradual thickening of the spindle fibres toward the equator may perhaps be interpreted as a differentiation initial to the formation of the plate.

The vitelline granulations, otherwise evenly distributed, are largely excluded from the stellar areas, and are less numerous about the animal pole than in the vegetative half of the sphere.

The phenomena connected with the production of the second polar globule are, from this point on, nearly a repetition of those of the first globule: the formation of the equatorial zone and its separation into halves; the translation of the whole figure along the animal radius; the ultimate attainment of the surface by the centre of the peripheral star; the consequent modification of its form; the deepening constriction which cuts down upon the spindle between the two lateral zones; the formation of a cell-plate; the gradual disappearance of the half-spindle in the polar globule, and a corresponding indistinctness in the vitelline half-spindle, — all these occur, with only slight modifications, in the manner already traced in the earlier stages. There are, however, some points worthy of more special attention. The second polar globule is generally *smaller* than the first. The relative sizes, which are subject to considerable variation, may be most easily comprehended by an inspection of the figures. Then, too, a certain obliquity of the parts about the animal pole, already alluded to (p. 182), is often observable during the formation of the second globule. This is well exhibited by Fig. 66. The internal half of the spindle is so obliquely placed as to appear almost parallel to a tangent at the animal pole; the long axis of the globule is also oblique, but is oppositely inclined, so that a sharp bend is caused in the course of the interzonal filaments. The centre of the deeper star thereby attains a more superficial position than would otherwise be possible, and it may be that we are to look to this fact for an explanation of the peculiar appearance. I am inclined to think, however, that the

obliquity is more likely to have been produced by an inequality in the constriction, just as in ordinary cleavage, where it is often found that the segmentation furrow advances from one side with much greater rapidity than from the opposite. In a view at right angles to this (Fig. 67) the spindle and globule are seen to be quite symmetrical, though not strictly radial to the vitellus. I have so often observed this obliquity, that, although certainly not to be considered as universal, I believe it to be characteristic of this stage.

Another not less peculiar, though by no means constant phenomenon, affects the inner star. During the formation of the second polar globule, the radiate appearance in the vitellus becomes wider and wider, until it at times is traceable to within a short distance of the periphery. It is at the close of the formation of the second globule that it seems to attain its maximum extent, — to dominate the whole vitellus. Lying as it does, with its centre so near the surface of the yolk, the rays are necessarily of very unequal length. That, however, is neither their most noticeable nor most interesting peculiarity. Seen in certain favorable positions, they are observed to stretch away toward the periphery, not in rigid straight lines, but in bold, sweeping curves, which are so related to each other that they present the appearance of extensive, more or less sharply curved spirals. A view upon the animal pole affords a survey of the most extensive curves. Occasionally (Fig. 78) the course of rays may thus be traced in a sweep of nearly 400°. Tracing them from the centre of the star, these fibres may be seen to curve in a constant manner, so as to be only slightly divergent. Finally, after completing an immense arc, they become invisible near the surface of the yolk. Such prominent fibres, however, do not describe their spirals in a single plane, but, as the focusing of the instrument as well as side views teach, they gradually descend toward the vegetative pole as they recede from the centre of the star. Necessarily not all the rays of a given vitellus are thus extensive, but all show the curvilinear course more or less distinctly. In some cases the rays do not seem to centre in a common point, but to arise along an axis, as in Fig. 66. The latter, however, is not a straight line, nor even a simple curve, but shares in the spiral influence expressed in the rays in such a way as to have approximately the form of a corkscrew. The dotted line $\alpha\ \beta$, Fig. 66, gives the *projection* of this corkscrew axis on the plane of the optical section.

Both the extent of the rays and the degree of their curvature are subject to great variation in different eggs. The opposite extremes to those just described are presented by Fig. 63, and all gradations be-

tween these extremes are to be met with. Nor does it appear that the *direction* of the spiral is constant, for, while my earlier observations chanced on cases in which the course was left-handed, later studies taught that the reverse was not uncommon, though probably of less frequent occurrence. Thus far I have not seen this spiral arrangement in the rays of the deeper star of the *first* archiamphiaster. On the other hand, the spiral rays of the outer star, already seen in the case of the first archiamphiaster, are often found in the second. No instance has come under my observation in which this arrangement was traceable in both stars of a given amphiaster.*

After the formation of the second polar globule, the influence which induces the stellate figure seems to quickly wane; for the rays become less and less extensive, and finally altogether undiscoverable, — a fact which may in some degree explain the differences of extent which prevail in the stellar figures of eggs otherwise presenting apparently the same stages of development.

The gradual disappearance of the deeper stellate figure is synchronous with other processes most intimately associated with the fate of the inner half of the second maturation spindle. Just as in the formation of the first polar globule, so here one half of the equatorial zone of fibre thickenings passes into the globule; the other half remains in the yolk, and moves along in the direction of the spindle fibres *toward* the centre of the deeper stellate figure. This centre, however, it does not reach in the condition of a group of thickenings, nor even soon after this form has given place to a more definitely circumscribed structure.

There is formed, at the expense of this inner zone, upon the completion of the second polar globule, a vesicular structure of irregularly spherical or ovoid form, which is at first homogeneous, or contains at most only a few highly refractive spherical bodies of unequal size. It is asserted that this structure is formed at the expense of the lateral zone of thickenings, not because a direct metamorphosis in the living egg has been observed, but rather as an inference from the fact that it occupies the place of the latter *near* the centre of the stellate figure when the zone as such has disappeared. Just what the relation of the individual thickenings to the ovoid vesicle is, I am not able to say. Either each becomes a centre about which is grouped a fresh accumulation of substance that ultimately unites with neighboring like masses to form the homogeneous contents of the vesicle, whose enclosed corpuscles (nucleoli) would then be identical with the spindle thickenings, or

* See p. 535.

the thickenings first unite to form a homogeneous mass, in which *new* bodies arise as nucleoli. A few facts seem to point to the latter as the more probable explanation. I have rarely seen a case, it is true, in which this vesicular structure was entirely homogeneous (Fig. 70^b); yet the small number of the enclosed bodies in the earlier stages, as well as their noticeable inequality of size, are facts not easily reconciled with the notion of a direct conversion of the thickenings into nucleoli. The constant increase in the number of these corpuscles indirectly favors the idea that *all* are new productions within the homogeneous nuclear mass.

For reasons which are already familiar to those acquainted with the phenomena of this stage of development, and which will be considered later in the present paper, this vesicular structure will be called the *female pronucleus* (*fpn*), and the contained bodies *female pronucleoli* (*fpnl*). The opacity of the yolk prevents observation on the living egg of the changes which accompany the origin of this pronucleus; so that the conclusions are necessarily of the nature of inferences, capable, however, of some degree of control. As previously stated, the earliest stage showing unequivocally the existence of this pronucleus is one in which pronucleoli are already present. But in some eggs, as in that represented in Fig. 63 (compare Fig. 70^b), a confused mass occupies the place of the lateral zone of thickenings, and may fairly be taken, I believe, as the incipient stage of this pronucleus, which does not as yet show any distinct traces of nucleoli. Treatment with osmic acid is generally much more serviceable for the discovery of this pronucleus than that with acetic acid. Possibly this may explain why some of the eggs subjected to the latter do not exhibit any distinct evidence of the lateral zone, or the pronucleus (Figs. 66, 67), when, to judge from other features, we might look with confidence for such structures.

At a later stage (Figs. 57–60) this female pronucleus is found still occupying a position near, but certainly not coincident with, the centre of the stellate figure. Its outline after treatment with acetic acid is quite distinct, and exhibits a double contour, which is usually more or less wrinkled (Figs. 57, 59). After treatment with osmic acid, however, the outline appears even, and there is no double contour to suggest the existence of a distinct nuclear membrane. In both methods of treatment followed by staining, the pronucleus is somewhat more deeply colored than the surrounding protoplasm; especially is this noticeable in the osmic acid treatment. By either method it contains a number of rounded bodies, varying from 2 μ to a minuteness bordering on the lim-

its of discernment with Hartnack, obj. 7, oc. 4. These contained bodies, or nucleoli, are very strongly refractive, and consequently appear as brilliant and conspicuous objects within the pronucleus, especially when treated with osmic acid followed by carmine staining. They are not always evenly distributed through the pronucleus, but are often grouped in different parts of its substance. No considerable portion of the pronucleus, however, is destitute of nucleoli.

The extent to which the inner star of the second archiamphiaster continues to hold sway in the vitellus is subject to variation. Often it is seen still at its maximum after the female pronucleus has acquired a diameter of 15 μ or 20 μ. At other times it is much less extensive (Fig. 63), even at the beginning of the formation of the pronucleus. The latter unquestionably continues to increase in size, and sometimes retreats a little from the animal pole of the yolk toward its centre; more often, I believe, it remains quite near the surface, — at the place it occupied when the confluence of the thickenings began.

Simultaneously with its increase in dimensions occurs an increase in the number of nucleoli. How the new nucleoli arise — whether by division of those previously existing or not — must, in default of direct observations, remain undetermined; yet the entire absence of forms showing any of the stages of division affords no support to the supposition of such an origin. There are, it is true, some deviations from this relation between the increase in the size of the nucleus and the increase in the number of nucleoli (Figs. 70, 77); but, in general, I think the correctness of the statement cannot be doubted. It is also quite certain that the size of the larger nucleoli is directly proportional to the size of the pronuclei themselves; so that a growth concomitant to that of the pronucleus may fairly be assumed. The nucleoli appear perfectly homogeneous, and very prominent in osmic acid preparations; in acetic acid, on the other hand, they are less distinct, and on the average somewhat larger. The outline, even with the latter method of treatment, remains full and entire, and in some cases it appears double; but nothing like a double contour is seen in preparations with osmic acid. Neither vacuoles, nor granulations, nor punctations, are discernible within the nucleoli by any method of treatment employed.

The changes which further affect the female pronucleus are rather those of growth than of migration. It is especially significant that this female pronucleus remains constantly near the surface of the vitellus. However much it may increase in diameter, its removal from the animal pole is never great, in most cases altogether inappreciable.

Owing to its increase in size, one of its margins may even come into closer proximity to the surface at an advanced stage than at an earlier one. Its relation to the centre of the vitelline star now in course of disappearance is hardly less interesting and important. It has already been stated that the female pronucleus is not *formed* at the centre of this stellate figure. It might be added, there is strong ground for believing that it never comes to occupy such a relation to the radiating fibres left in the vitellus after the detachment of the second polar globule. Certainly, in advanced stages of its formation (Figs. 57, 59, 68), this centre of radiation is distinctly outside the boundary of the pronucleus, — to say nothing of the coincidence of their centres, — and often at a considerable distance from it. It may be here remarked concerning the last trace of the second archiamphiaster, that the rays of its internal star fade away so gradually throughout their whole length that it is often quite as difficult to distinguish them near their point of convergence as at any other part of their course. Compare Fig. 59.

The diameter of the female pronucleus may eventually attain one fourth the diameter of the whole vitellus (Figs. 73, 74, 77), or even in some cases a third of its diameter (Fig. 85).

When treated with acetic acid its shape is extensively modified by deep and numerous wrinkles and folds which have a very characteristic appearance. The outlines are usually more or less concave outwardly, as though caused by the thrusting outward of some angular contained body; yet the protruding points are not sharp, but close to the apex become rounded, so that really there are no "cusps" formed by adjacent curves, as one is inclined to think at first sight (Figs. 59, 80, 85, etc.). The outline becomes more irregular and wrinkled in advanced stages of the nucleus. In this method of treatment, too, the outline constantly appears double, and increases in distinctness in proportion to the size of the nucleus. Within the latter, one distinguishes in advanced nuclei a large number — up to fifty or sixty — of nearly spherical bodies, the pronucleoli, which often exhibit double contour lines. I have never seen one of these nucleolar bodies sufficiently different from the others in size, or behavior with reagents, to warrant the distinction of a main and accessory nucleoli; and only once (Fig. 52, chromic acid preparation) have I seen anything like a nuclear reticulum.*

When treated with osmic acid and subsequently stained in carmine,

* P. S. In eggs of an undetermined species of Limax I have observed in both female and male pronuclei a single nucleolus of much greater size and more deeply stained than the other nucleoli. Compare Figs. 80^b, 80^c, and explanations.

the female pronucleus presents a different appearance, one which in some respects doubtless reproduces the natural condition more truly than the acetic acid process, though in other points it may be doubted if it does not offer quite as much violence to nature as the latter. The pronucleus, far from being wrinkled, presents a most delicate and even outline, which seldom deviates from a continuous and often exquisite curve. It often approaches a spherical form; at other times it is ovoid, or may even be pear-shaped (Figs. 68, 77, etc.).

From a large number of comparisons, the conclusion seems reasonable that, treated in this manner, the pronucleus, though very likely approaching quite nearly the form and proportions which it had in the fresh condition, nevertheless has suffered an absolute reduction of size much greater than in the other method of treatment, and somewhat greater proportionate diminution than the vitellus. The same, too, is doubtless true of the pronucleoli.

No trace of a double contour can be found by the osmic acid method, either for pronucleus or pronucleoli. The substance of the nucleoli, as well as that of the pronucleus in which they are imbedded, appears perfectly homogeneous with the highest power used. (Hartn., obj. 9, oc. 4.)

The nucleoli, as before, appear to vary somewhat in size, but are uniformly much more prominent than when treated with acetic acid. They present a rounded form, but do not approach so closely that of the sphere as in acetic acid specimens, and they show the same want of regularity in arrangement that has already been noticed.

In proportion to its service in making prominent the nuclear structures, osmic acid fails to be of value in making distinct the radiations of the vitellus which characterize different stages in the development. Some traces of the stellate figures may usually be made out, but they are never present in that unequivocal boldness which belongs to acetic acid preparations.

The female pronucleus often lies in such close proximity to the surface of the vitellus that the latter becomes involved in its extensive foldings, and consequently shows corresponding wrinkles and depressions (Fig. 73). In a more marked degree something of a similar nature has taken place in a few of the specimens treated with osmic acid (Figs. 69, 75). In these cases, however, there is not a general wrinkling of the pronucleus. The nearest portion of the vitelline surface appears as though forced inward like a hollow plug, thus causing a corresponding depression in the outer half of the pronucleus.

Sometimes there is a similar plug-like projection from the opposite or

deeper face of this pronucleus, which in turn impinges on a second similar body. The origin and destiny of the latter will form the subject of subsequent considerations. Thus, in optical section, one or both of these pronuclei appear to have the shape of a crescent, with blunt rounded horns. In these cases, caused, probably, by too prolonged or vigorous action of the acid, the contents of the pronucleus are altogether homogeneous, and without any trace of nucleoli, notwithstanding the size of the pronuclei. I am at a loss to explain this disappearance of the pronucleoli. There is only one, not altogether satisfactory explanation that has occurred to me. I have already stated my conviction that the nucleus becomes more contracted by treatment with osmic acid than when hardened in acetic acid. This diminution of size may best be accounted for on the supposition that it suffers a loss of fluid components, — becomes more concentrated, and compact. If such an assumption is legitimate, it is at least possible that the above-described condition may have been brought about by so prompt and considerable a loss of nuclear fluid as to make the substance of the nucleus very compact and refringent, — so refringent, in fact, as to leave no perceptible difference between the nucleoli and other parts of the substance of the nucleus. Another possible assumption is, that the nucleoli were already dissolved in preparation for the coming metamorphosis into a nuclear spindle.

Éd. Van Beneden (**'75**, p. 698) has observed something which I am inclined to think is very like what I have described, if not indeed identical with it. I am not able to speak with the greatest certainty, since the description referred to is not accompanied by figures, but I am the more inclined to think the phenomena are identical, because Van Beneden in this case also employed osmic acid. I shall recur to this point again.

II. Fecundation.

In eggs examined soon after extrusion there are to be observed in the vicinity of the vitellus a number of small ovoid bodies, which are at once noticeable from their possessing considerably greater refractive power than the surrounding albumen. (Fig. 49.) These bodies are of even outline, about 10 μ long and 7 to 8 μ wide. The greater number usually lie near the vitellus, some apparently in contact with it, while numbers are scattered irregularly in different parts of the albumen. Among these may usually be found some which have a filamentous structure protruding from one side. On further inspection it will appear that the remaining portion is of the same nature, but from the closeness of the

coils the filament is easily overlooked, except when the uncoiling is already begun. At a somewat later stage the uncoiled filaments outnumber the oval bodies, and still later few or none of the latter are to be found. The oval bodies are unquestionably single coiled spermatozoa which suffer an unfolding and at length lie in the food-mass as outstretched male elements. They are often wrapped about the vitellus, at other times thrown into irregular curves in the surrounding albumen. The unfolding must be slow, for I have repeatedly watched in order to discover the nature of the process, but have never succeeded in seeing motion, either in the oval bodies or in the outstretched spermatozoa. I have never failed to find spermatozoa when made an object of special search. In some cases they are present in great quantities, even forming extensive trains through different parts of the albumen.

Neither the growth nor the structure of the spermatozoa has been made the subject of extended observations. As regards their form it may be seen from Fig. 94 that they are thread-like, gradually tapering from immediately behind the "head" to the opposite extremity. They may attain a length nearly equal to the diameter of the vitellus. The "head" is flattened, oval, somewhat pointed at its free end, and when seen sidewise appears tongue-shaped and joined to the neck at a very slight angle. The portion following the head has often a wavy course, while the terminal part frequently remains in a loop.

I once saw quite distinctly an interesting spermatozoön in the albumen of an egg already treated with reagents, and made at the time (Aug. 15, 1878) a hasty sketch, which I have had reproduced in Fig. 94, since it indicates the existence of an *undulating membrane.* So far as I am at present aware, this has never been observed before of the spermatozoa of slugs. Leydig (*Lehrbuch d. Histologie*, p. 533) says that hitherto (1857) zoösperms with undulating membrane have been found only in the cases of Rotifers and Cypridæ. Whether we have to do in the case of Limax with dimorphic forms of the spermatozoa, I cannot say, as I have given the subject no attention. The spermatozoön here figured was motionless, so that I can only *infer* that the membrane figured undulates in the active spermatozoön, after the manner known in the salamander and other animals. The loop at the end of the tail in this case seems to be very delicate; perhaps it is formed exclusively by the thin membrane.*

* Sept. 1, 1880. Gibbes ('80) has recently demonstrated the existence of the vibratile membrane in the case of several vertebrates, and some invertebrates. Among the latter is Helix; I am thus the more confident that it is really an undulating membrane which I have seen in Limax.

No motion in the spermatozoa having been seen, their direct penetration into the vitellus is necessarily beyond the observer's experience; nor has anything been observed in the living egg at this early stage to indicate that penetration had taken place. Nevertheless it is highly probable, judging from the observations that have recently been made in cases more favorable for the study of this phase of development, that the substance of at least one of these thread-like spermatozoa is already embraced within the vitellus at the time the egg is laid.

The earliest indication observable in the living egg which can be referred even indirectly to the presence of a spermatozoön occurs some time after the formation of the second polar globule.

Allusion has previously been made to the fact, that after the formation of what has been called the female pronucleus there appears a second structure of similar aspect. The latter is situated at quite a distance from the former, and is often more deeply imbedded in the vitellus, on account of which it is usually less distinct (m. pn., Figs. 65, 30). After a time it is found nearer the female pronucleus, and in proportion as it nears the latter it becomes larger and more easily seen. At length the two lie side by side, but still continue to increase in size. I have not been able to make out any constant difference in dimensions between them; it is therefore often quite impossible to say which is the female pronucleus, and which the other body, unless they have been under observation for some time previous to their contact. The latter has quite the same appearance as the female pronucleus. Its study in the living egg is permitted only occasionally by an exceptional transparency of the yolk. Under favorable circumstances its outline is seen to exhibit slow changes of form such as have already been described for the female pronucleus. In its growth it keeps pace with the latter. In some cases considerable portions of their surfaces appear in contact. In others, however, even when of great size, they are only very close to each other, and do not touch.

The meagre results to be obtained from the study of the living egg are very well supplemented, in some points at least, by that which may be learned by other means of investigation. The use of osmic acid is more satisfactory than any other single method. I have not had the same success in the use of acetic acid, since it does not cause the vitellus to become as transparent, nor are the nuclear structures made so conspicuous by it. To the fact that osmic acid was used much less frequently than acetic acid is probably due my want of success in detecting this nuclear body at an earlier stage in its formation.

In no case have I found any evidence of the existence of this body before the formation of the second polar globule. However, there is always to be found in hardened eggs, as soon as the female pronucleus has become well marked, another structure resembling the female pronucleus (Figs. 58, 60), which is usually located at some distance from the animal pole.

It ordinarily has almost exactly the same size as the female pronucleus, and deports itself quite the same as the latter, when treated with different reagents. From its position it is unquestionably the deeper clear spot discovered in the living eggs. In the earliest condition in which it has been satisfactorily observed, it has, with a single exception (Fig. 70^b), already attained a considerable size, and contains a number of highly refractive spheroidal bodies. It is found at this stage (Fig. 60) still not far removed from the surface of the vitellus. It becomes visible in the *living* egg only at a later period, when it has altered its position and attained greater dimensions.

From its subsequent changes and ultimate fate there need be no hesitancy in calling it at once the *male pronucleus* (*mpn*), even though its relationship with a spermatozoön could hardly have been surmised, but for the very conclusive observations made by several observers within the past few years, — one might almost say months. In a few cases toward the close of the constricting phenomena, which set at liberty the second polar globule, and before a distinct female pronucleus had appeared, I have noticed, very close to the surface of the vitellus in the vegetative hemisphere, small vacuoles (diam. 6 μ) of homogeneous appearance (Fig. 66), which I am inclined to consider as earlier stages in the existence of the male pronucleus. The preparations were all such as had been produced by the use of acetic acid and subsequent staining in Beale's carmine, which, as before stated, is less favorable for making conspicuous the pronuclei than is the osmic acid process. The interference of the granular elements of the yolk is such as to make the observations on these small structures exceedingly difficult and little reliable, for which reason it may be better to consider the object seen as only possibly bearing the interpretation suggested. The doubt as to the significance of these structures was further increased by the fact that in one case — the one figured — *two* such vacuoles of almost identical appearance occurred in remote parts of the same vegetative hemisphere. It might not be absolutely impossible, it is true, that these vacuoles resulted each from the presence of a spermatozoön, but it would be highly improbable, in the light of all that has

recently been learned concerning the relation of egg and spermatozoön. In one other case, alluded to above, a perfectly homogeneous oval vacuole (Fig. 70^b, β) was observed near the surface of the yolk at the equator. The female pronucleus of the same egg (Fig. 70^b, α) was of nearly the same size, but contained a single large nucleolus. In all cases the vacuole seemed filled with a substance of less density than the surrounding portions of the vitellus.

As already indicated, the male pronucleus in its more advanced stage is so nearly similar to the female pronucleus in all morphological points, that it would be mere repetition to describe it in detail. Not only the size of the two at any given time, but also the form, the characteristic behavior with different reagents, the appearance of the nucleoli, even the number of the latter, are subject to such unimportant differences that it would be quite impossible for one, however familiar with them, to say which was the male and which the female element, were it not for the positions which they occupied relative to each other and the remaining parts of the vitelline sphere.

Inasmuch as there exists a definite relation between the size of these pronuclei and their distance apart, — which may be expressed by saying, the larger the pronuclei, the nearer they will be to each other, — it might be justly inferred, even without the corroborative evidence of direct observation in the eggs of many other invertebrates, that migration of one or the other of them takes place. From what has already been said of the migration of the female pronucleus, it may at once be inferred that this approximation takes place principally, if not exclusively, by a change in the position of the *male* pronucleus.

When within a short distance of each other, the two (Fig. 68) are often seen with their more pointed ends directed toward the centre of a stellate figure. In case acetic acid has been used, the form becomes much altered, and it is no longer possible to observe so clearly this condition; but even here their mutual relationship to the stellate figure may often be very easily demonstrated (Figs. 57, 59).

Notwithstanding that in many cases this position may be shown, even in an advanced state of the pronuclei, in other cases the female pronucleus appears to be more or less coincident with the central portion of the stellate figure (Fig. 72), although it probably never happens that the *centres* of the two structures exactly coincide. In reality this aster often becomes quite invisible before the close approximation of the two pronuclei has been effected.

There may then be left for a time an irregular area in its place, where

the coarser granular elements of the yolk do not intrude (Fig. 70), but subsequently the force that kept these granulations back seems to yield completely, and no part of the vitellus remains absolutely free from them.

A condition such as is presented by Fig. 68 might leave one in doubt whether this aster belonged to the male or the female pronucleus. A comparison with numerous other cases (e. g. Fig. 72) leads me to think there is no room to question its being an archiaster, — the remnant of the inner star of the second archiamphiaster.

When it has attained considerable size, and is consequently in the vicinity of the animal pole, the male pronucleus may sometimes be seen in the living egg. In some cases, either from the comparative absence of yolk granules, or from its superficial position, or from both, it may be easily distinguished as a sharply marked spheroidal body (Figs. 21, 30), although nucleoli are not always distinguishable. In fact, both the male and female pronucleus — the latter more superficial, and the former somewhat deeper — were seen and figured in Limax as long ago as 1850 by the Russian naturalist Warneck (**'50**, Taf. IV. Fig. 10'); although the state of embryological science at that time did not allow this very accurate observer to interpret his observations as successfully as may be done to-day.

The pronuclei ultimately come in contact, and their increase in size does not seem to cease when they touch. They become more or less flattened against each other, but an actual union, as observed in the case of many other animals, is not to be seen here. If it ultimately takes place, as we must conclude it virtually does, the union is so late and so involved in other phenomena as to become entirely unrecognizable as such. It is, therefore, at this point that the egg of Limax presents one of its most interesting and instructive phases. Before proceeding to consider these changes, which belong strictly to the process of segmentation, it is desirable to say a few words concerning some appearances which must doubtless be considered abnormal. Although the observations are meagre, a brief statement of them may be welcome, since, as far as I am aware, no one has recorded similar observations concerning the eggs of Limax, nor indeed of any of the Mollusks.

For one interested in recent observations upon impregnation the description of the *male pronucleus* cannot fail to be of interest in a negative way, inasmuch as no allusion has been made to the existence of any special arrangement of the protoplasmic substance immediately surrounding it. It has been ascertained by several observers, that, in many animals, the male pronucleus early becomes, if not the centre, at least the

region of a special stellar structure, which extends in all directions from the pronucleus, and which has a separate origin from all the stellate structures connected with the production of the polar globules. To this star has been given the name (Fol, **77**[a], p. 360) *male aster.* It therefore seems remarkable that this structure, which I have called the male pronucleus in Limax, should not be accompanied by some trace of the characteristic star. This would have afforded grounds for apprehension lest my interpretation of this nuclear body might be erroneous, were it not that the structure of the body, and its deportment under the influence of reagents; its growth, ever parallel to that of the female pronucleus; its migration toward, and finally its contact with, the latter, pointed unequivocally to its nature as the male element. In no case, by whatever method treated, was any trace of such a stellate structure in the protoplasm surrounding the male pronucleus to be detected, either in its earlier or later stages, although carefully sought for in all the numerous specimens of this age which have come under my observation.*

It is, therefore, a matter of interest, that a single egg (Fig. 81), which must probably be considered abnormal, should afford the only trace of what is so common to the male pronuclei of eggs in other animals. In this specimen, beside an extensive system of delicate rays which centres near an irregular body that may possibly be the female pronucleus, there are no less than half a dozen other systems of rays, varying somewhat in size, distributed through the protoplasm of the vitellus in such a manner as not to be very close to one another, nor yet very near to the surface of the yolk. Each of these smaller stellate figures is composed of a few prominent short rays, directed toward a central, homogeneous body of small size. After studying the observations of Hertwig and Fol (**77**[c], p. 469), it cannot be doubted that this is probably a case in which numerous spermatozoa, instead of a single one, have effected an entrance into the egg, and that their number must have been at least as great as that of the observed smaller stars, whose central corpuscles may therefore be considered incipient male pronuclei. In the immediate vicinity of the largest aster are to be found two or three other nuclear elements, of which certainly one, and possibly two, occupies the centre of its own special system of rays, now considerably obscured by the predominance of the larger system with which it has become confused. In the case of the nearer one, the appearance is much as though a union with the female pronucleus were being effected. The

* The only possible exception has been explained above, p. 220.

latter exhibits no indication of nucleoli.* The egg was treated with acetic acid, and subsequently stained in Beale's carmine.

III. Cleavage.

The living egg has been followed in its changes through the formation of two polar globules and the subsequent growth and approximation of two nuclear bodies, the so-called male and female pronuclei.

These nuclei remain near the surface at the animal pole. They may be distinguished in the living egg as two distinct bodies up to within a short time previous to the rapid changes of the first cleavage. Shortly before that event, their outlines are no longer discoverable in the fresh egg. The region remains more clear, but all that can be distinguished is a more or less circular, ill-defined area, which is less opaque than the surrounding portions of the vitellus. After a few moments, this area grows less distinct. It finally appears elongated. Very soon this lengthening has resulted in two light spots, which are inconspicuous at first, but which increase in size and distinctness, and at length become oval (Fig. 31). The long axes of the ovals are so directed that, if prolonged, they would meet a little way beyond the animal pole of the yolk.

During the earlier part of this series of proceedings, — viz. soon after the meeting of the two pronuclei, — there is usually an accumulation of transparent protoplasm about the animal pole; or, in other words, the granules of the vitellus vanish from this portion of the yolk, leaving sometimes a very thick superficial layer (Fig. 70[a]), at other times only a comparatively thin covering, of clear protoplasm. This surface layer may occasionally be traced quite around the yolk; in other cases, it can be followed for only a short distance from the animal pole.

If the outline of the egg be carefully watched about the time of the formation of the two new light spots, it will be seen gradually to lengthen in a direction corresponding to the line which joins the spots (Fig. 31). As the latter enlarge, the lengthening increases, though not very conspicuously. At length a slight flattening of the surface appears just under the polar globules. This finally changes into a very shallow depression (Fig. 37), which grows deeper (Fig. 32), and becomes angular. If the yolk be viewed along the polar axis at this time, it will

* It is possible that the body I have called male pronucleus *(mpn ?)* may represent the female pronucleus, in which event the structure marked *fpn ?* might be only the "area" of the deep star of the second archiamphiaster.

be seen to be considerably flattened parallel with the plane passing through the animal pole and the two spots. The latter appear in this position transversely oval.

A little later, the furrow will be seen to have extended around on the sides of the yolk as a shallow depression, reaching something more than half-way toward the vegetative pole. The oval spots have meantime been increasing in extent, and their axes have now (Fig. 32) become more nearly parallel. In some cases one may see a faintly but unmistakably radiate arrangement of the vitelline substance around these oval spots as centres (Fig. 32). More frequently the yolk is too opaque for that, or even for the detection of the oval spots. Soon after the appearance of the depression at the animal pole, — within four or five minutes at the usual temperature of a summer day, — the furrow has run quite around the yolk, and now appears at the vegetative pole as a very broad, shallow depression (Fig. 35). This annular constriction now deepens on all sides, but most rapidly from that of the animal pole. Thus the axes of the two clear spots become first parallel, and then somewhat divergent toward the animal pole. But when the furrow of the animal half has advanced to near the middle of the yolk, it has become narrowed, by the approach of the opposing faces of the incipient spheres, almost to a fissure (Fig. 62[a]), whereas the depression from the opposite side is now a broad groove, so that the axes of the two clear spots have by this means become again convergent toward the animal pole. By the further deepening of the constriction on all sides, there are formed two equal, symmetrical, ovoid bodies, which are connected by only a slender thread of protoplasm, situated much nearer the vegetative than the animal surface (Fig. 62[a]). The long axes of the new spheres are at this moment (before complete separation) directed convergingly toward a point in the plane of division which lies in the prolongation of the animal radius. At this time, too, the blunter end of the new spheroids is the one corresponding to the vegetative half of the unsegmented yolk. Seen along the animal radius, they appear elongated in a direction perpendicular to the line joining their centres, and the surfaces which face are less convex than those which look outward. A plane perpendicular to that of cleavage, and coinciding with the animal radius, would divide each into symmetrical halves. There is no other plane which could accomplish a like result.

At length the slender filament becomes more attenuated, and finally parts. The cleavage is accomplished; but each of the spheroids still continues to undergo further changes of form, and promptly assumes

new attitudes towards its fellow.* Alterations within the yolk are not easily observable. The light spots grow somewhat less distinct, but further than this nothing can be seen.

In returning to the study of the normal course of development of *hardened* eggs, I begin at the point where its consideration was left for the purpose of presenting the abnormal conditions shown by the presence of a number of spermatozoa in a single yolk.

In the eggs of most animals the pronuclei, after attaining their maximum size and coming into close contact, suffer a mingling of their substances by the disappearance of the limiting envelopes (membranes) that for a time separated them. This union becomes so complete that authors speak of the resultant structure as a single body, — a unit, which has been called the nucleus of the first segmentation sphere. In the case of Limax, we find that this unit structure is sought with very questionable success. *The first cleavage nucleus does not have a morphological existence.* I seek an explanation of the fact by assuming that the acceleration at this stage of the ontogeny is so great that the division of this promised structure is begun before it has an actual independent existence. To say the least, the first *evidences* of the coming separation of the yolk have already made their appearance, while there are still two distinctly separate pronuclei. I refer in these evidences to the *new* stellar shapes which arise in the protoplasm of the yolk in the vicinity of the two pronuclei, and which are destined to become the amphiaster of the first cleavage sphere. Undoubtedly, the two new centres of activity which now come into existence are only a part of a continuous process of transformation which neither begins nor ends with them, — a process which slowly obliterated the great spiral archiaster, and which will in turn cause them to disappear; but it is equally certain that they belong strictly to the phenomena of the first segmentation. In their beginnings, these new centres exert an influence which, though not far reaching, is vigorous and aggressive. All observations hitherto agree in making the visible changes connected with these two centres synchronous in their appearance; and this may well be true for the majority of cases. It, however, is not universally so, for in several of my preparations (Figs. 52, 73, 79, 80) very satisfactory evidence is afforded that one of the new centres may exert an influence of considerable extent before its mate has produced the slightest visible sign of its existence.

Usually these new stellar figures centre at points on, or very near, the

* The mutual flattening of the products of segmentation, and other interesting phenomena of the same period, cannot be considered in the present paper.

surface of one or both the pronuclei. Most observers locate the two points very definitely: both stars are made to lie in the plane along which the two flattened pronuclei are at first in contact and then confluent, and they are represented as occupying two diametrically opposite points in the circumference of that plane. From this it follows that the new centres are made to lie *in the surface of the new nucleus* (or nucleus of the first cleavage sphere). However it may be in other cases, it no longer holds good as a distinctive position for Limax. Unexceptionally, there is a more or less marked antipodal relation expressed in the position of the two new centres; but *they are not uniformly in contact with, nor even in close proximity to, either of the pronuclei* (Fig. 85), and when such approximation does exist (Figs. 52, 74), it may be that *one of the new centres is in relation with only one of the still separate pronuclei.* So great an ontogenetic concentration as the contemporaneous existence of the *archi*aster and one or both of these *new* asters has never been observed. The question may arise whether the single centres of radiation above mentioned (Figs. 52, 73, 79, 80) may not have been the last remnants of archiasters, since the latter persist for a long time. I think the answer may be most positive that they are not; for the archiaster fades gradually in all parts, and if the central portion remains visible a trifle longer than the rest, it is only as a very indistinct structure. But the asters which concern us now are vigorous, though not yet of extensive influence; they are sharply marked by rays of comparatively limited extent. Furthermore, their positions are not favorable to such an interpretation. They are uniformly nearer the middle of the vitellus than are the centres of the pronuclei. There is, however, some variability in the closeness of the latter to the surface of the yolk, which is manifest in both fresh and hardened specimens. The two light spots seen in the living egg, just prior to and during the first segmentation, appear from their positions to coincide with the asters.

The condition of the pronuclei at the time of the origin of these new centres and during their increasing ascendency is of special interest. As has been already indicated, it is very difficult to prove in eggs treated with acetic acid that there is a direct union of the pronuclei. Even in cases where the amphiaster of the first cleavage sphere has acquired a considerable extent (Fig. 85), it is clear that the two pronuclei have not become fully amalgamated into a single structure, and it may possibly be questioned if any portions of their substance have become confluent. The nucleoli, though more faintly outlined than in an earlier stage, are still easily recognizable.

At somewhat earlier stages (Figs. 73, 74, 79, 80), the nucleoli are found to be very numerous. As many as sixty have been seen in a single pronucleus, but generally about thirty are embraced in each of the pronuclei. The variations in size are considerable in the same pronucleus, and the average size is often noticeably different in those of different eggs.

Both pronuclei continue to exhibit a double contour (when treated with acetic acid) even after the appearance of the asters of the cleavage sphere. The outline is much wrinkled (Figs. 73, 74, 79, 80, 85). Usually at the time of the first appearance of the new asters the pronuclei are close together, and it is difficult, by reason of their foldings, to determine how extensively the two bodies are in contact. In a few cases there could be no doubt in the matter, as the nuclei were in contact at only a single point, or not at all. The specimen shown in Fig. 79 must have been hardened very early in the formation of the new amphiaster, as only one of the asters is visible. In the more advanced stage represented in Fig. 85, the extent of the contact is certainly much greater, and the distinctness of outline along the contiguous faces so much impaired as to leave the impression that a fusion of nuclear substances has already begun. In the vicinity of this plane of contact there are to be seen in each of the pronuclei a few highly refractive granules much smaller than the pronucleoli, and not arranged in any discoverable order. Aside from these two sorts of contained structures, the contents of the pronuclei remain, as before, homogeneous.

The first cleavage amphiaster, in the earliest conditions seen, consists either of a single stellar figure, or of two, quite limited in extent. The rays are fine and approach the common centre so closely as to leave only an exceedingly small area of homogeneous appearance; in some cases, indeed, it is impossible to make out such a definitely circumscribed portion. The absence of a distinctly marked area is not confined, however, to the early stages of the amphiaster. Eggs present in this respect individual differences, so that in one the area may be seen with great distinctness, and in another of the same age be indistinct; even in the same amphiaster one aster may show no line of demarcation about its central area, while that of the other is sharply limited. The rays, which at first are quite uniform in prominence, have been seen, at stages a little later (Figs. 85, 82), to be differentiated in such a manner that one side of the star is more conspicuous than the other. Whereas in the earlier condition the central area appears spherical, in the more advanced condition it is flattened in the direction of the line joining the halves of

the amphiaster. The central portion of each aster thus becomes somewhat lenticular in form. Its outline is less convex on the face, which looks toward the remaining aster of the pair. More or less in conformity with this change in the shape of the stellar "areas," the asters themselves are modified from the perfectly spherical appearance which they at first present. The rays are no longer of the same length, nor are they all uniformly tapering. At some distance from the centre is disposed a more or less complete zone of much thicker and more conspicuous fibres (Figs. 85, 82). These are not thickened throughout their whole extent, but are abruptly enlarged. The enlargements are continued for a distance equal to the breadth of the zone, when they are as abruptly reduced to the ordinary dimensions. These zones are not of uniform prominence on all sides of the aster, but in places gradually fade away.

The central area of each stellate figure may remain for some time homogeneous (Fig. 85, *a a*); at length there appear within it, however, groups of highly refractive granules (Fig. 82), which, with the flattening of the area, assume a corresponding distribution, so that when seen in profile they have an irregular linear arrangement. When the stellate figures have attained this complication of structure (Fig. 82), the pronuclear bodies are no longer recognizable as distinct structures. All that is left to indicate their previous existence are numerous dark granules irregularly arranged midway between the two asters, and, in a region that is now traversed by stellar rays, a few very faint circular outlines (*pnl ?*) of a size corresponding to that of the pronucleoli in the stage just preceding. The indistinctness of these circular outlines prevents that certainty which one feels in regard to the existence of the dark granules, and this mistrust is increased by the appearances presented by other eggs (Figs. 86 – 89), where there is found a trace of the nucleus of the first cleavage sphere, but where there is no evidence of any contained nucleolar bodies. Notwithstanding this apparent contradiction, I am inclined to believe that occasionally the pronucleoli may in part persist even after the disappearance of the outline of the pronucleus, and after its substance has become so diffused as to be no longer readily distinguishable from the surrounding vitelline substance.

The dissolution of both these structures — the pronuclear membrane and the pronucleoli — doubtless falls within a very limited extent of time, and it may not be wrong to infer that in one instance the disappearance of the one precedes, whereas in another case the disappearance of the other first takes place. A trace of the nuclear substance has even been

found at a somewhat later period; at a time, namely, when the spindle of the first cleavage sphere is fully formed (Figs. 88, 89), and possessed of equatorial thickenings.

The remnants of the nuclear structure sustain such a topographical relation to the forming amphiaster and spindle as to leave little doubt that the latter take their origin nearer the centre of the vitellus than the place occupied by the pronuclei, so that the substance of the pronuclear bodies moves from its superficial position toward the centre in contributing to the formation of the spindle. The opinion that such a motion of nuclear substance as here suggested actually takes place, may find support in the shape often presented by this nuclear remnant. It seems to be elongated toward the amphiaster, and sometimes shows (Fig. 87) a sort of filamentous structure, as though individual fibres of substance were being drawn into the forming spindle. These nuclear remnants are flattened in such a manner that they are seen edgewise when one looks along the axis of the forming spindle. As a consequence, they are more conspicuous when viewed in this position than when seen *en face*. In the latter case the outline is much less regular, and in places may be quite indistinguishable; especially is this the case along the border directed toward the amphiaster. For this reason it is not easy to satisfy one's self as to the exact direction in which the nuclear substance is tending. Seen edgewise, it is unequivocally directed toward the axis of the spindle. But toward which part of the axis, — toward the middle, or toward one or both of its apices? This can be satisfactorily answered only by a study of the face view. Though not so satisfactory as the former aspect, this view favors the belief that the nuclear substance is being transferred toward the *equatorial* region of the spindle.

The spindle is formed some time after the first appearance of the stellate figures. It is only in an advanced stage of the metamorphosis that the existence of such a structure, distinct from the general radiation about the centres of the two asters, becomes evident. I have seen very delicate and inconspicuous fibres stretching from star to star *outside* the pronuclear structures at the early stage represented in Fig. 85. These, however, are not distinguishable from the neighboring rays of the asters, unless it be by a somewhat greater length. The centres of the stars lie deeper (farther from the animal pole) than the adjacent surfaces of the pronuclei, so that if spindle fibres are present, as I believe, they must lie external to the pronuclei. Even in the later stage represented by Fig. 82 the limits of the spindle

are not satisfactorily indicated. In other cases, even before the entire disappearance of the nuclear structure (Fig. 86), a portion of the rays form a continuous, thick, spindle-shaped body, whose fibres converge toward the centre of the asters. These interstellate fibres, however, are not traceable to the centres of the stars, but are lost in the margins of the central "areas." The limits of the spindle are much more clearly indicated a little later, when equatorial thickenings appear. Occasionally, however, the arrangement of the thickenings in the equator is very irregular (Fig. 92). I believe, but cannot say with certainty, that this indicates an early condition, and that it is followed by a more regular arrangement. Usually the equatorial thickenings are so uniformly disposed that their combined effect is the same as though resulting from a flat disk occupying exactly the equator of the spindle (Fig. 89). The thickness of the latter is somewhat more than half its length (Figs. 83, 89). An optical section corresponding to the equatorial plane (Figs. 84, 92) shows that the thickenings in the case of this amphiaster, as in that of the archiamphiasters, are arranged in the form of a ring, so that it may be inferred that the nuclear fibres are themselves more numerous near the surface than at the axis of the spindle.

The changes in the general outline of the vitellus, which have already been noticed in speaking of the living egg, are preserved in those treated with acid. The first change — a lengthening of the axis of the yolk which is parallel to the spindle occurs at about the time the equatorial zone of thickenings is formed. This prolation of the yolk is quite apparent, as in Fig. 89.

A comparison with views along the animal radius, however, shows that this change of form is as much due to a flattening at the animal pole, and consequent shortening of the corresponding axis, as to a lengthening in the direction of the spindle. In such a view, either from the animal or from the vegetative pole, the outline of the yolk may remain circular, even when the spindle is completely formed (Fig. 83).

Up to this time the stellate figures have been constantly increasing in size, though not uniformly on all sides. The rays are traceable for the greatest distance in planes passing through the centres of the stars, and parallel with the equator of the spindle. More than half the rays of each aster are found on the distal sides of these planes. Each of the asters is substantially hemispherical. The rays now stretch out to near the periphery of the yolk, and approach each other along the equatorial plane. The latter thus becomes apparent as neutral territory, where

the forces inducing the stellar figures have not extended their influence, or serve to hold each other in check. It is along this plane that the deepening fissure passes which ultimately separates the yolk into two equal spheroids.

But while these changes in the form of the yolk are in progress, the spindle undergoes modifications like those which transpire in the maturation spindles. The equatorial zone of thickenings splits into lateral halves, which migrate each toward the corresponding apex of the spindle. Such, at least, is the inference to be drawn from the fact, that, after the flattening and during the subsequent constriction of the yolk, the equatorial zone is wanting, and in its stead two lateral zones are found, which are farther from the equatorial plane the deeper the constriction. That the migration of these thickenings is comparatively rapid may be inferred, I think, from an examination of Fig. 90; the separation of the two lateral zones there exhibited having been accomplished between the beginning of the depression at the animal pole and the appearance of a shallow furrow at the opposite side of the yolk. When seen exactly edgewise, the lateral zones present a linear arrangement of the thickenings parallel to the equatorial plane, but a slight deviation in the direction of sight causes each lateral zone to assume an oval outline, one edge of which appears, however, by careful focusing, a little deeper than the other. This is represented in the drawing by making the deeper half of the outline less distinct.

As the constriction advances from the animal pole, the interzonal filaments (*Kernfäden*, Strasburger) which were left behind by the separating lateral thickenings, are forced before it, and thus become bent so that their convexities are directed toward the vegetative pole. This bend retains for some time the nature of a full uniform curve on the convex side, but on the indented side it soon assumes a more angular appearance (Fig. 93). The interzonal filaments thus continue to be carried forward by the advancing depression of the yolk without surrendering their continuity. In this manner they become lengthened, inasmuch as the position of their extremities remains comparatively uninfluenced by this change. They thus form a sort of V-shaped figure, whose free ends terminate in the lateral zones of spindle-fibre thickenings. In all this process the interzonal filaments appear to play an entirely passive *rôle*. Meantime the lateral zones have assumed the nature of nuclear vacuoles containing each a number of nucleolar bodies. Whether this has transpired by the accumulation of nuclear sap in the region of the thickenings, now converted into nucleolar bodies, or has resulted from a

confluence of the thickenings in which new nucleolar structures have arisen, I am unable to determine. The fact that no cases of entirely homogeneous nuclei have come under observation, does not seem favorable to the latter hypothesis, and yet the same method doubtless prevails here as in the formation of the female pronucleus. Treatment with acetic acid has invariably resulted in a shrivelled appearance of the nuclei. With the deepening of the constriction from the animal pole, the two nuclei assume a lengthened form, and take positions such that their long axes correspond approximately with the trend of the interzonal filaments. Toward the close of the constriction (Fig. 91) each of the nuclei has attained a length of a quarter to a third the diameter (ca. 35 μ) of the resulting cleavage spheres. Its breadth is not more than half its length. It is somewhat more convex on the face which looks toward the animal pole. Double contour lines are more manifest with increasing size. Both the number and magnitude of the nucleolar bodies increase. They now number twenty or thirty. They vary in size from 3 μ or 4 μ to less than 1 μ, and are nearly spherical.

When the constriction has advanced from the animal pole to the centre of the yolk, the interzonal filaments are most conspicuous exactly opposite the constriction, where they appear somewhat thickened. These thickenings, however, are never abrupt, but taper gradually on both sides, till, in the vicinity of the nuclei, the filaments become quite faint. As the constriction advances further, this indistinctness affects more and more the terminal portions, till at length only very short, rapidly tapering threads are seen. These, however, persist for some time; they may even be discovered near the surface of the yolk after the two spheres have become detached from each other, and have entered upon other phases of relationship. These thickenings are doubtless equivalent to the so-called "cell-plate" of Strasburger (*Zellplatte*). The last point of union between the halves of the first segmentation sphere is marked by this remnant of the interzonal filaments; it is they which are last to yield to the force that divides this first cell into two.

There remains still another point to be mentioned in connection with the phenomena presented by eggs hardened during the process of cleavage. It often happens that this treatment causes a thin, superficial layer of the yolk to become separated from the rest of the vitellus on that side where the constriction is farthest advanced (Figs. 90, 93). This appears as a homogeneous structure, less than 1 μ in thickness. The inner surface is less sharply marked than the outer, and from the fact

that it is only incompletely differentiated from the underlying granular protoplasm it is found in places to have portions of the latter clinging to its inner face. From this I can hardly conclude otherwise than that a differentiation has already begun in the superficial portion of the yolk, which is the first step toward the formation of a cell membrane, and that this differentiation is proportional to the advance of the cleavage. It is unquestionably owing to the action of the acid that this layer becomes detached from the vitellus; but it seems to me unnatural to conclude that the homogeneous layer has itself been produced by the action of the reagent.

B. BIBLIOGRAPHY.

I. Limax.

The embryology of Limax has been studied by a number of naturalists. The most of the papers on this subject, however, were written many years ago; indeed, no extensive contribution to the embryology of Limax has appeared for the last quarter of a century. The phenomena which are considered in the present paper were in many cases either briefly touched upon, or fell altogether outside the province of the authors' investigations. Of the latter class, and consequently of less immediate concern at present, are the papers of J. L. M. Laurent (**'37, '37**[b], **37**[c], and **38**[a]); P. Laurent (**'42**); Dujardin (**'37**); Schmidt (**'51**); Gegenbaur (**'52**); and Lankester (**'74** and **'75**[a]).*

1. *Egg Envelopes, etc.*

The composition of the egg at the time of extrusion has been studied by several writers, whose conclusions, though not difficult to interpret, are somewhat at variance.

The earlier descriptions date from a time when the process of segmentation was as yet unknown in Mollusks. It would be unreasonable to expect from them valuable observations on many of its structural features.

Turpin's (**'32**, p. 435) description is substantially as follows. The eggs of Limax flavus (Fig. 9) are oblong, terminated at each end by a sort of umbilical chord; they are transparent, bluish or grayish, soft and gelatinous. The eggs of the Limaces are composed, like those of Helices, of

* The numbers in Egyptian type following an author's name are abbreviations for the year of publication, — e. g. **'37, '37**[b], **'37**[c], all appeared in 1837, — and serve at the same time to refer the reader to the alphabetical list of authors quoted.

four parts: two envelopes, an albuminous liquid, and a cicatricula. The exterior mucous envelope, which is quite thick and resistant, is distinguished by a sort of loose network composed of very delicate fibres. The interior envelope, of extreme thinness, hyaline, and likewise furnished with a fibrous network, contains the albuminous liquor and the cicatricula. The eggs of Limax rufus appear entirely like those of Limax flavus.

The figure given by Turpin is not such as to aid materially in understanding the structure. His exterior envelope includes both the external stratified and the homogeneous layers, while his internal envelope is the *membrana albuminis*, whose wrinkles were probably mistaken for a fibrous network. The cicatricula is the yolk.

The description given three years later by J. L. M. Laurent of the eggs of Limax flavus and of those of "Limace rouge," differs from that of Turpin in only two or three points.* Passing from without inward, Laurent (**'35**[b], p. 249) found in succession: —

1. A mucoso-corneous shell, evidently formed of concentric layers.
2. An internal membrane.
3. Two albuminous layers, the more liquid enveloping the denser one.
4. A very small vitellus, whose color, a slightly yellowish gray, varies with the incidence of the light.

No other author, so far as I know, has observed any differentiation of the albumen into a denser and a more fluid portion. His "internal membrane" doubtless corresponds to the *memb. albuminis*, and his "shell," the concentric layers of which are first mentioned by this author, embraces, like that of Turpin, the homogeneous as well as the stratified portions. In a subsequent paper, accompanied by a plate, this author (**'38**, pp. 155, 333, and Pl. 3) states that between the corneous shell and the internal membrane there is a clear space, which is traversed by fibrillæ (which in his Fig. 1 show a reticulated arrangement) joining these two structures. A transparent, watery fluid may accumulate in this clear space. It probably corresponds with the viscid, unstratified shell layer of later observers. It is possible that Turpin saw some such network of fibrillæ, and hence ascribed to the whole of the outer thick envelope this structure. I do not find it mentioned by subsequent observers, nor have I seen any such peculiarity myself.

* The eggs of L. flavus are united in a chaplet; those of "Limace rouge" are smaller and isolated.

It is possible that Laurent had observed the so-called chalaza at the time (1835) his first article was published; but it was subsequent to the appearance of a paper by Van Beneden and Windischmann, — in fact after that paper was already known to him, — that he speaks (**'38**, p. 134, foot-note), for the first time, of a "filament tortillé qui existe constamment dans tous les œufs de limace." This is described more at length on page 146 of the last-mentioned paper.

Although Dumortier (**'37**) states in his memoir on the development of Mollusks that he has studied the eggs of Limax, his results are all drawn from the study of Lymnæus ovalis.

In the same year (1838) that the last-mentioned paper of Laurent appeared, Van Beneden and Windischmann (**'38**) wrote of Limax agrestis as follows: "As regards the composition of the egg, we have found almost nothing which does not accord with the observations of M. Laurent. Nevertheless, we have established the presence of a 'cordon filamenteux' which becomes especially visible at a certain epoch of development, and which appears to us to have an evident analogy with the chalaza of the eggs of birds. This same cordon has already been pointed out, however, in the eggs of Helix pomatia."

In their final paper (**'41**, p. 6 [p. 20, *Études*], and **'41**[a], pp. 178, 179), published in 1841, these authors announced the following as the composition of the egg, from within outwards: 1st, a vitellus; 2d, a great quantity of albumen holding in suspension the "filament entortillé" (previously called "cordon filamenteux"); 3d, a delicate, transparent membrane covering the albumen; 4th, a slight layer of liquid; 5th, a quite thick exterior membrane composed of numerous layers. The points of correspondence with the descriptions of Turpin and Laurent are apparent. The clear space of the latter author is the liquid layer of Van Beneden and Windischmann. The structure called "filament entortillé" was at first (1838) regarded by Van Beneden and Windischmann as homologous with the chalaza of birds' eggs, but, from the variations occurring in different eggs, they subsequently concluded that it was a torn membrane which at first surrounded the vitellus, and that consequently it really appertained to the vitelline membrane. The latter view has been sufficiently refuted by Warneck (**'50**, p. 107).

The description of the egg by the last-mentioned observer (pp. 105–111) differs in some points from that of his predecessors. The albumen is invested by two membranes, — *membrana albuminis secundaria seu interna*, and *membr. alb. primaria seu externa*. The former is easily thrown into folds by compression; the latter is much thicker than the

former; both are structureless. External to the latter is a layer of viscid mucus (*Schleim*), which fills all the space between the external membrane and the stratified, elastic shell. In approaching the deeper portions of the layer this mucous substance increases in consistency.

I have been unable to discover the membrana externa, and believe that the structure so named by Warneck may be only a somewhat denser portion of the mucous layer, which does not always differ enough in refractive power from the remaining portions of the mucus to make it distinguishable.

Warneck (**'50**, p. 108) says that crystals of lime carbonate are found on the outer surface of the outer shell, in some places united into a druse; and subsequently Gegenbaur (**'52**, p. 372), evidently without knowledge of Warneck's paper, reported that one very frequently sees in the *middle strata* of the layers of the outer shell deposits of the same substance in the form of dark round concretions, which also often assume a crystalline structure. I have seen nothing of the kind.

2. *The Yolk and its Changes.*

The authors who have studied the early condition and changes of the yolk of Limax are J. L. M. Laurent, Van Beneden and Windischmann, Warneck, and Gegenbaur.

Very little is to be learned from Laurent (**'35**[b], and **'38**, p. 136), further than that he believed the vitellus might vary considerably in form, and that it appeared to embrace a variable number (15–20) of large globules containing smaller ones, — views which show clearly that he is more likely to have had under observation segmenting eggs than such as had not reached the cleavage stage.* In view of this fact, not much importance attaches to his statements when he informs us, that a central whitish spot, situated more or less closely to the circumference, is visible by reflected light, and may be due to reflection of the light alone (!); that he has never succeeded in recognizing the least indication of a *cicatricula* produced by the liquid of the germinative vesicle; or that he thinks there is a vitelline membrane.

From what has already been said of the view entertained by Van Beneden and Windischmann (**'41**[a], pp. 179–181, Taf. 7) as to the nature of the "filament entortillé," it will be clear that they inclined to the belief that the freshly deposited egg was without a vitelline membrane. All the eggs observed by Van Beneden and Windischmann had

* The process of segmentation in Mollusks, as is well known, was first announced by Sars (**'37**, p. 402), in 1837.

been deposited for a longer or shorter time. In keeping with the then prevailing opinion of the total disappearance of the germinative vesicle, they report not being able to find a trace of it at the centre of the yolk. The first change observed was the appearance of a transparent vesicle, which seemed to escape from the midst of the yolk, and was soon followed by a second. These vesicles (polar globules) are never wanting, and always escape from the same side of the yolk. After their escape, the space which they have traversed appears clearer (deep aster ?) than the rest of the vitellus (sufficient cause for them to institute a comparison with the vase-shaped portion of the white yolk in the fowl's egg). The vesicles contain granules some moments after their escape; they take no part in the formation of the embryo, but are subsequently absorbed in the albumen. The observers are unable to say whether these vesicles have any analogy with the germinative vesicle, but think it in no way astonishing that the latter should escape from the yolk, if the vitelline membrane has suffered the change above alluded to, although the vesicle would in that event cease to have the important (germinative) *rôle* attributed to it in higher animals.

What is said concerning the first segmentation becomes intelligible only by comparing with the figures, and apparently rests on a misconception of the order of events. It is stated (p. 181) that, after the escape of the two vesicles, the middle of the vitellus becomes clearer, and is divided into two equal portions, and that subsequently a furrow makes its appearance at the side opposite that where the (polar) vesicles escaped. An examination of Fig. 5 (Taf. 7), which is described (p. 194) as representing the stage in which the yolk has become clearer in the centre, shows conclusively, to one who has seen the object itself, that the clearness in question is due to a lense-shaped accumulation of a transparent fluid between the cleavage spheres,* and that consequently the figure represents a stage *after* the first cleavage. I am unable to explain how it happens that the cleavage furrow should be considered as first appearing at the side *opposite* that where the polar vesicles arise (see Fig. 7). That the process was not very carefully observed is moreover sufficiently demonstrated by this Figure 7, for it unquestionably represents an egg *after* the first segmentation, and after the accumulation of the lenticular mass of fluid above alluded to.

Warneck (**'50**, pp. 102, 103, 105, 114) thinks that the yolk in the eggs of Lymnæus and Limax has no special envelope, but is simply

* This interesting phenomenon has been in most points already well described by Warneck, and has been seen in other than molluscan eggs.

clothed in a layer of soluble protoplasm (*Schleim*) which takes the place of a membrane. It possesses an envelope (*Hülle*), it is true, but it is not membranous, — it is simply one of thickened protoplasm. For this reason the author cannot agree with those who do not admit the existence of any envelope.

The observations of Warneck on the early changes of the egg in Lymnæus and Limax were far more extensive and accurate than those of his predecessors; they were, in fact, in advance of most of the contemporary studies in other branches of embryology, and came near anticipating some of the more important discoveries of the present decade. His paper (1850) marks approximately the beginning of a reversion of ideas as to the total disappearance of the Purkinjean vesicle which had been dominant since its discovery in 1825; and, if I have not mistaken his meaning, Warneck may fairly be reckoned among the first who entertained doubts as to a complete dissolution of this structure.

It must be admitted, however, that the reputation of Johannes Müller was the sufficient cause for the more general reception of new views on this point in embryology. Although Warneck's paper antedates by two years that of the latter author, there can be little question that the influence of his writing has been inconsiderable when compared with that of Müller.*

According to Warneck (**'50**, pp. 114, 115) one discovers within the yolk-mass of the impregnated egg a clear spot, which is due to a cavity filled with an albuminous fluid as clear as water, and containing no elementary corpuscles, such as occupy the yolk. This clear spot occupies exactly the place of the Purkinjean vesicle. No distinct contour is observable, the transparency of the spot diminishing toward the periphery, so that it gradually merges into the yolk-mass. Its position is central.

Although not thus sharply formulated, I think there can be no doubt that Warneck believed the "clear spot" to be the Purkinjean vesicle metamorphosed by the disappearance of its membrane and its numerous germinative dots (p. 177).

* Among others, Hermann Fol has recently called attention to this paper by Warneck, whose work he estimates very highly. While one cannot fail to admire the generous spirit which prompts this opinion of the merit of Warneck, it is much more difficult to subscribe to the interpretation which Fol puts upon certain portions of his paper.

Brandt (**'77**[b], pp. 593, 594) has made use of the studies of Warneck to corroborate his own observations on the amœboid nature of the germinative vesicle, in a manner which appears to me unjustifiable, as will presently be shown.

The changes affecting this clear spot are briefly as follows (pp. 116–118). The spot, which at first appears quite round from whichever side viewed, becomes lengthened in the direction of a diameter, and assumes successively the forms of a biscuit (*Semmel*) and of a figure **8**, and finally becomes fully separated (i. e. differentiated) from the remaining yolk-mass.* This process is one of segmentation or division. The constricted "spot" migrates toward a definite point of the periphery. The end which the contents of the spot approach, becomes considerably enlarged, so that the clear spot assumes the form of a blunt rounded cone.† If the egg of Lymnæus vulgaris arrived at this stage be crushed, it will be found that the spot really consists of two globular parts surrounded by a transparent protoplasmic substance (*Schleim*) which considerably increases the size of the spot. The very thin envelope (*Hülle*) of the spheres contains a transparent albuminous fluid, of the same nature as the surrounding protoplasm (*Schleim*). In the case of Limax agrestis the phenomena are substantially the same. The two spheres become readily distinguishable, especially when one of them moves outward (through the yolk).‡

* "Der Anfangs vollkommen runde Fleck verlängert sich in der Richtung des einen Durchmessers, nimmt darauf eine biscuitähnliche Form an, dann die Form einer 8 und zuletzt trennt er sich ganz von der übrigen Dottermasse."

Another rendering of this passage would be, "and finally becomes completely *eliminated* from the remaining yolk-mass." There is also one other passage which might possibly support this view. I shall presently quote and endeavor to explain the second passage. The objection to accepting any interpretation which admits that the spot is eliminated, is the positive denial of it subsequently made by Warneck.

† "Das Ende, welchem sich der Inhalt des Fleckes nähert, vergrössert sich bedeutend, so dass der helle Fleck die Gestalt eines stumpfen, abgerundeten Kegels annimmt."

‡ As the passage last alluded to (Warneck, **'50,** p. 118) seems to have been the cause of some misunderstanding, I will give it in full: "Es bilden sich also im hellen Flecke bei Limax auch zwei Kugeln, welche besonders deutlich unterschieden werden können, wenn die eine von den Kugeln nach aussen tritt, wie es gleich beschrieben werden wird."

The interpretation of the author's statement turns on the meaning of "nach aussen tritt." I understand by it the same as would have been expressed by saying "nach aussen — nach der Peripherie zu — tritt." Had it been the intention of the author to say that one of the spheres was eliminated, he would have used "heraustreten."

The difficulty of understanding what is meant results from the addition of the clause, "wie es gleich beschrieben werden wird." There is nothing in the subsequent description to which this allusion can refer, except the formation of two polar globules. It is probably on account of this that Warneck has been understood to

The description thus far evidently relates to the formation and migration of the structure called in the present paper the *first archiamphiaster*, and to the method of its production from the clear spot which succeeded the Purkinjean vesicle. Although revealing none of the finer structural changes which have recently been brought to light, this description leaves no doubt as to the nature of the object, nor the substantial accuracy of the observations. The same cannot, however, be said regarding some of Warneck's subsequent statements. One does not, at least, feel the same certainty that the author has before him this archiamphiaster phase of development when he says (p. 119) that the difference between Limax and Lymnæus consists only in this, that each sphere in the case of the latter possesses a thick envelope with two distinct contours. This statement does not agree with that already cited, declaring the spheres to have a thin envelope. The discrepancy is probably due to Warneck's having confused the asters with pronuclei, in which event the case of spheres with double contour must have been such as is really found only at a later period, viz. after the formation of the polar globules. It seems unreasonable that so accurate an observer should have made such a mistake, and yet I do not see any other possible explanation. It would be entirely unreasonable to suppose that the archiamphiaster in Lymnæus possessed double contour lines, even if the convincing studies of Bütschli on this genus were wanting. What is said relative to the vesicles found in these nuclear spheres may well be referred to the pronucleoli.

In Warneck's opinion (pp. 121, 122) these two nuclear spheres remain quite passive during the formation of the polar globules, which takes place from the pole of the yolk toward which the spheres have migrated. This process is described at some length.

Fol, it is true, finds authority in Warneck for saying that one of these nuclear spheres escapes under the form of globules, — the excretory (or direction) corpuscles.* The only passages allowing such an interpretation are the ones already quoted, but the subsequent statements of Warneck are so entirely unequivocal that I cannot hesitate in believing his conception to have been quite unlike that which Fol ascribes to him. Warneck says (p. 120) that there appears between the outer rim (i. e.

say that the polar globules are produced by the elimination of one of the nuclear spheres. On the other hand, he states, in the most positive manner, that the polar globules have nothing to do with these nuclear spheres. (See below.)

* "Cette tache se divise en deux moitiés, dont l'une reste au centre du vitellus, tandis que l'autre arrive à la surface, et sort sous forme de globules : les corpuscles excrétés (ou de direction)." (Fol, '75[a], p. 22.) Compare also Fol, *op. cit.*, p. 26.

the base) of the conical spot and the envelope of the yolk a clear crescent-shaped place, which can be plainly distiguished from the clear spot and the remaining yolk-mass. A little further on, he says the crescent-shaped space shows quite clearly that the nuclei do not in the least take part in the formation of the outer vesicles (polar globules).*

If this understanding of Warneck be the right one,† it will be seen that his observations on the archiamphiaster are not only very incomplete compared with recent studies, but that they are radically at fault in making this structure and the polar globules *independent* of each other. It might at first seem somewhat strange that no evidence of radiate structure should have come under his eyes, inasmuch as he certainly made use of acetic acid; but the probable use of a concentrated acid, and the absence of any staining process, — which latter is really of very great additional value, — are doubtless sufficient to explain this oversight.

First there appears, then, according to Warneck, a clear crescent-shaped place between the outer rim of the conical spot and the protoplasmic envelope of the yolk, which can be clearly distinguished from the clear spot and the remaining yolk. This is erroneously considered by Warneck as identical with a most interesting and peculiar

* The words of Warneck (pp. 121, 122) are as follows: "Der sichelförmige Raum, welcher die ursprüngliche Erhabenheit [of the polar globule] von dem hellen Flecke trennt (d. h. den beiden Kernen mit ihrer durchsichtigen, eiweissartigen Hülle), zeigt ganz deutlich, dass die Kerne nicht im Mindesten an der Bildung der äusseren Bläschen Theil nehmen. Daher muss ich mich durchaus gegen die Ansicht erklären, nach welcher die Bläschen aus dem Centrum der Dottermasse entstehen sollen, oder, was einerli ist, dass diese Bläschen als vesicula Purkinji oder als Ueberbleibsel derselben zu betrachten seien. Ich bin ganz überzeugt davon und meine Abbildungen zeigen dieses ganz deutlich, dass die Hülle der Bläschen ursprünglich mit der Dotterhülle ganz gleichbedeutend, und der Inhalt derselben aus dem sichelförmigen Raume entlehnt sei."

† P. S. — Since writing the above, I have chanced upon Bütschli's (**'76,** pp. 242, 243) interpretation of Warneck, which I had overlooked. Bütschli's conclusion is substantially the same as that to which I have arrived, and he also finds himself compelled to pronounce Fol's interpretation wrong, without, as it seems, having discovered the two passages which make the chance of such a mistake possible. It seems a little strange, in view of this criticism by Bütschli, that Fol still maintains the same estimate of Warneck's meaning touching the relation of the archiamphiaster to the polar globules. Fol (**'79,** p. 149) writes as follows: "Le cône transparent prend une forme plus évasée et sa partie superficielle donne naissance, par une sorte de bourgeonnement, à un globule polaire, puis à un second, et rarement encore à un troisième." Perhaps the passage in Bütschli's work has escaped the attention of Fol, as it at first did mine.

phenomenon which reappears with each segmentation; viz. the accumulation of a transparent fluid secretion, which becomes periodically evacuated into the surrounding albumen (compare pp. 131–136, *l. c.*).

In this stage there are produced out of this crescent two vesicles whose emergence begins by the protrusion of a small elevation (*kleine Erhabenheit*) through the protoplasmic envelope of the yolk. The elevation increases, assumes the form of the segment of a sphere, then that of a hemisphere, which gradually becomes a complete sphere resting on a rather stout pedicel. By a constriction of the latter, the sphere appears as a free vesicle. The statement that the second appears *gleich* after the formation of the first, cannot be taken as exactly representing the facts. The first globule is larger than the second, and, while the former contains in its clear albuminous fluid small elementary granules, the latter contains a nucleus and nucleolus. Warneck also observed, though very rarely, a third vesicle. The globules do not take part in the formation of the embryo, but continue to exist in a collapsed condition till the young slug escapes from the egg-shell.

For Warneck these vesicles have not the great importance implied in the name "Richtungsbläschen." The coincidence of the place of their appearance and that of the first traces of segmentation does not establish the dependence of the latter on the former. The views of Bischoff, Kölliker, and Reichert, in referring these vesicles to the germinative vesicle, are especially denied, as far as relates to the snails; and, as we have already seen (p. 240), their connection with the "clear spot" is denied with equal emphasis; from all which I believe it clearly follows, as stated above, that Warneck entertained the opinion that a genetic connection did exist between the Purkinjean vesicle and the "clear spot." *

Warneck's interpretation of the physiological signification of the polar globules hinges on the supposed identity of their formation and detachment with the later phenomena of the elimination of a clear liquid. Of the vesicles themselves, he says, they in all probability remove from the yolk an albuminous fluid (p. 123). Of the later phenomenon he says (p. 134), "Die untauglichen Stoffen werden auch bei dieser Art der Thätigkeit durch Exosmose entfernt."

From a comparison of the text (p. 125) with the descriptions of the figures (pp. 180, 181) we learn that the elimination of the polar globules

* P. S. — Again in this point I cannot agree with Fol ('79, p. 145) when he cites Warneck in the following connection: "Dans l'embranchement des Mollusques, la disparition du noyau de l'ovule a été reconnue par de nombreux observateurs. . . . . Je citerai les travaux . . . . de Warneck pour Limnæus et Limax."

is followed by important changes in the yolk. The crescent-shaped space disappears. The "clear spot" passes from the conical to an oval form, then becomes figure-eight-shaped, and finally appears as two distinct nuclei, which are distinguishable from the earlier condition by reason of the sharp contour of the walls, and the presence of a large nucleolus in addition to less prominent vesicles. These nuclei have an eccentric position; they lie nearer the point whence the polar globules were detached.

There can be no question about these nuclei being really the male and female pronuclei. I have not, however, been able to discover in either of them in the case of Limax campestris a single nucleolus of greater size and refractive power than the other contained bodies. The contour of these nuclei (pronuclei), says Warneck, grows indistinct, their envelopes are dissolved, and the contents of the two form a single mass, which, before the division of the yolk, becomes oval, and then biscuit-shaped, with the long axis at right angles to the position previously (in the conical clear spot) occupied (nucleus of first cleavage sphere ?).

What Warneck has said of the cleavage of the yolk, as observed in the living egg, is of less immediate interest, and from its substantial agreement with the description given in the first part of this paper may be passed with a few words upon a single point. Warneck says (p. 128) that the plane of the first cleavage furrow is not perpendicular to the long axis of the yolk, but cuts it at an angle of almost 45°. I have sometimes seen such an obliquity in the course of the furrow, though never so great an angle, nor do I think it can be true for anything like a majority of cases with Limax campestris.

I return to his account of the clear nuclear spot. With the lengthening of the yolk, and its constriction, the spot diminishes to one fourth its former dimensions, and is only visible with difficulty, especially in the case of Lymnæus.*

There can be no doubt, he very rightly affirms, about the dissolution of the nuclear membrane and the nucleoli. This, and the failure of the nuclear substance to curdle when exposed to water, lead him to the conviction that this clear spot has again altered its chemical properties, and

* The somewhat incongruous statement that, for Lymnæus, this obscurity arises, as he *thinks*, from the fact "that the contents of the nucleus mingle with the remaining yolk-mass, and that the spot (*Fleck*) withdraws itself to the centre of the yolk at the formation of the dorsal [i. e. at the animal pole] furrow," could not have outweighed in his own mind the direct observations of a division of the spot in the case of Limax.

that this must be the cause of its further metamorphoses. Finally, during the division of the yolk, the clear spot becomes fully divided into two parts, each with a trail, like the tail of a comet, stretching away toward the last point of contact. The trails soon disappear, and the spots gradually assume the globular form, though not yet capable of being isolated from the yolk, from which he concludes the nucleus has not yet a distinct membrane.

Accompanying the subsequent approach and mutual flattening of the cleavage spheres * the nuclei acquire a membrane. That Warneck had observed very closely the formation of the new nuclei must be evident, I think, from the following passage (p. 138): "Im Anfange . . . . sind sie [die Kerne] noch sehr klein (noch kleiner sogar, als diejenige helle Masse, welche in jede Kugel sich absondert) und fast glaube ich, dass nicht die ganze Masse unmittelbar in jede Kugel [jeden Kern?] übergeht, sondern nur ein gewisser Theil derselben wird Anfangs, gleich einem Centrum, von einer Hülle umgeben und vergrössert sich dann auf Rechnung der sie umgebenden Masse."

Gegenbaur (**'51**, pp. 373, 374) has only incidentally touched upon some of the questions which interest us, his main purpose being the investigation of the genesis of the organs and their tissues. The absence of a cell membrane, owing to the essential importance at that time attached to this structure, seems to have caused him to entertain a less just conception of the cell nature of the cleavage spheres than was held by Warneck; for he remarks that only a negative result was obtainable in regard to the question of the cell nature of these spheres, inasmuch as he was in no way able to demonstrate a membrane, and therefore concludes that these spheres are to be considered as in the process of being generated into cells, — "als angelegte Zellen," — which only attain their formation into genuine cells after oft-repeated division.

The so-called "Richtungsbläschen" arises through the elimination (*Ausscheidung*) of a little drop of yolk substance, which often contains various globules of fat, and often remains a long time in the vicinity of the yolk without acquiring any further relation to the embryo except that

* Of the phenomena which are observable during or immediately subsequent to this flattening, already very well described by Warneck, I will limit myself here to the statement that in Limax campestris the elimination of the secreted fluid takes place at the animal pole.

I would also say concerning a subsequent phase of segmentation, that in L. campestris the stage consisting of 8 cleavage spheres is not followed by one of 12, but both large and small spheres divide at the same time, so that the stage of 16 spheres follows directly that of 8.

it always arises from that part of the yolk where the constriction subsequently appears. The statement that "Grösse sowie Anzahl ist sehr differirend," can only have been the result of a superficial study of these bodies.

P. S. — Two papers on early stages in the embryology of Limax and Helix respectively, one by Mayzel and the other by Pérez, will be considered in an Appendix.

II. Review of Maturation, Fecundation, and Cell-Division.

1. *Cell-Division.*

The significance of the now well-established cell theory is hardly more far-reaching than is that of its legitimate offspring: the discovery, on the one hand, of the substantial identity of *cell-division*, whether in the animal or the vegetable kingdom, whether at the beginning or at the close of that cycle of events which makes up what we call the life of the individual; and, on the other hand, the growing conviction that *fecundation* is the reunion of forces which have suffered a complementary differentiation in cells whose corresponding parts become directly commingled in this act.

As the influence of the cell theory on investigations for the past forty years can hardly be overestimated, so we may confidently look forward for no mean outcome from the impetus imparted to biological research by these more recent achievements. The influence of studies on cell phenomena culminating in such broad generalizations, if less significant to the popular mind than evidences of a process of evolution drawn from the structure and habits of adult beings, is none the less securely intrenching the belief in the consanguinity of all living things.

Of all the phenomena connected with cell-division, that which may be designated as the metamorphosis of the nucleus has recently received most attention. It is that which has remained almost up to the present time superficially observed, not at all understood, and therefore the cause of many conflicting statements. Some portions even of the more obscure changes which take place within the cell during the nuclear metamorphosis were long ago seen, though hardly comprehended. These observations were for the most part limited to the changed appearance of the protoplasm surrounding the nucleus, — the stellate figures, — and were made on one or the other of the two cellular elements of sexual reproduction. Previous, then, to the consideration of the metamorphosis of the nucleus, the observations on the stellate

figures made prior to the time (1873) when they were shown to be intimately connected with the nuclear changes will be historically reviewed.

In order to study the *changes* which take place in the nucleus during cell-division, it will also be desirable to review late studies and opinions on the nature of the quiescent nucleus, — if we may speak of an apparently less active condition as a quiescent state. This will necessitate a second digression from the main topic, now under consideration.

a. Asters.

Stellate arrangements of the protoplasm surrounding the nucleus were observed long ago, but it is very questionable if many of these earlier observations were really made on the stellate figures which accompany cell-division. Especially doubtful are those descriptions in which a distinct nuclear structure is made the centre of an extensive radiation in the protoplasm. For the fully formed, conspicuous nucleus does not in most cases correspond to the stage in which the radial arrangement is prominent. In fact, with the completion of the nucleus, the radiate structure usually vanishes altogether. The representation of rays about a nucleus is not enough, then, to warrant the conclusion that an author has observed the astral figures which accompany division. To accept such as sufficient evidence is to ignore an almost constant relation of the astral centre to the nucleus.

One of the earliest and most striking cases of such a radial phenomenon is that described and figured by Carus ('**32**, pp. 44, 45, Taf. II. Figs. 3, 10, 11). It is true Carus regards, though erroneously, the embryos (Unio) figured as presenting abnormal conditions resulting from death, yet the nucleus and the radial structure of the surrounding protoplasm are so clearly shown that at first one would not hesitate to pronounce his figures to be those of veritable asters. So strong as the resemblance is, it must, however, be admitted that it is at best only a resemblance, and not a true aster. The latter does not exist when the nuclei present the appearance reproduced in Carus's figures. Flemming does not hesitate, after a study of the same objects, to declare the radiation in this case to be purely the result of a play of fancy.

The figures accompanying the work of Grube on Clepsine ('**44**, Taf. III. Figs. 11, 12) would lead one to suspect that he might have seen the radial arrangement of the protoplasm in the periphery of the nuclei, his "Wandungskugeln"; but I do not find that he makes any mention of it in the text, and am by no means sure that the radiate lines may not be due to something entirely different, possibly to fissures caused by excessive or unequal hardening of the eggs. At least, his figures do not present as acceptable evidence that such phenomena were seen as do those which Derbès ('**47**, Pl. V. Figs. 4, 6) executed three years later in giving the embryology of the urchin (Echinus esculentus). Derbès's first-mentioned figure is that of the egg after the disappearance of the germinative vesicle,* but before the first segmentation. From the

* Derbès, it is true, did not regard his "sphère moyenne" as a germinative vesicle.

central position of the nuclear structure it is to be inferred that it is either the female pronucleus, or, more probably, the primary segmentation-nucleus. As no description of it is given, it is not possible to say with certainty which of these structures it represents. The author says (p. 90, *op. cit.*) that after the yolk divides the first time he has seen that each of the resulting segments contains a small vesicle, and each of the vesicles is the centre of a slightly confused radiation. At the next subsequent segmentation he did not discover the vesicle, but only the radiation around a more or less centrally situated point, and in subsequent segmentations even this radiation was no longer perceptible. Even in the case of the divided yolk, there is no evidence that Derbès saw an *amphi*aster, or any part of the spindle.

In the same year (1847) similar phenomena were observed in the "Brutzellen," from which are produced the male elements of reproduction, — the spermatozoa. Reichert (**'47**, pp. 120, 121, Taf. VI. Figs. 1, 23, 24) was, so far as I know, the first to discover this peculiarity of the sperm-cells, which he describes in the case of a Nematode, Ascaris acuminata. After the division of the parent cell into two, four, or more sperm-cells, each of the latter presents a very peculiar and elegant radiate appearance in its central granular portion, which constitutes the principal part of the cell, and is surrounded by only a narrow transparent zone without granules. The central portion of this granular mass is, says Reichert, more translucent than the peripheral, owing to the presence of a nucleus. The centre of the nucleus, whose outline it is difficult to see, is occupied by a dark round spot, the nucleolus. Immediately around the latter (therefore *inside* the limit of Reichert's nucleus) the fat granules (Fettkörperchen) look like little dots, but toward the periphery they are of increasing length, so that the outermost present the appearance of little rods, all pointing toward the centre of the cell.

In the excellent studies of De Quatrefages (**'48**, p. 177, Pl. III. Fig. 11) on Hermella is contained an allusion which is probably referable to a central stellate figure, whose delicate rays, however, remained undistinguished.

Krohn (**'52**, pp. 314, 315) seems to have been the first to notice an *amphiaster*, although he did not, after all, make special note of any connecting structure between the two stars. In artificially fertilized eggs of Phallusia mammillata, he observed that at the approach of each segmentation the clear vesicular nucleus disappears, and that in its place there is found in each segmenting sphere a very peculiar arrangement of the yolk molecules. These granules, namely, dispose themselves in thick-set streaks (dichte Streifen), which appear to arise from *two* centres of radiation (Irradiationscentren), whence they are directed outward toward the lighter periphery. By the time the nuclei have made their appearance within the new cleavage spheres, the radial streaks have disappeared.

The paper is without accompanying figures, but the description is so accurate that it leaves no doubt as to the real nature of the rays.

A few years later, Reichert's observations were confirmed by Meissner (**'54**, p. 209, Taf. VI. Fig. 1), who found the radial structure both in the parent cells

(Keimzellen) and daughter cells, from which the male elements of reproduction are developed in Nematodes. In Ascaris mystax, A. marginata, A. megalocephala, and A. depressa, the same phenomena were observed. After the disappearance of the nucleus of the parent cell, the granules retreat a little from the cell wall, and begin gradually to assume a very uniform radial arrangement, like acicular crystals emerging from a somewhat clearer common centre. There is no nucleus in this centre, but the granules there are fine. (This whole granular portion Meissner erroneously considered to be the nucleus, Kernmasse.) The centre of radiation, hitherto single, becomes indistinct, and gradually a double or multiple arrangement makes its appearance, while the nucleus becomes constricted. The centre of radiation moves continuously toward, and finally attains, the centre of the new nuclei (Kernmassen), which furnish the basis for the formation of the corresponding cells.

The passage in REMAK (**'55**, p. 132) cited by Whitman (**'78**[a], p. 16) as evidence of the former's observation of a radial arrangement of the yolk substance in the segmenting eggs of Rana, does not seem to me as convincing as one might wish. That Remak himself had no such conception of the nature of the appearances figured (Taf. IX. Fig. 2, *op. cit.*) must, I think, be tolerably evident from the context.* The passage (compare Taf. IX. Fig. 2 of Remak's work) in question (§ 10, p. 132) is as follows: "Legt man die Hälften des Eies in diesem Zustande [i. e. during the first segmentation] aus einander, so erkennt man an beiden durchaus gleiche Beschaffenheit der Innenflächen: zunächst eine centrale runde oder ovale Bruchfläche von körnigem Gefüge und von wechselendem Umfange, so zwar, dass sie den grössten bis herab zum kleinsten Theil der Innenfläche einnehmen kann. Die scharfe Grenze der Bruchfläche wird durch den Uebergangsrand der beiden Scheidewände gebildet: sie macht sich durch die Glätte und Färbung der letzteren leicht kenntlich. Im Bereiche der unteren Eihälfte sind die Scheidewände durchaus weiss oder weissgelblich, also in ihrer Farbe nur wenig von dem angrenzenden Zooplasma (Dotter) verschieden, dagegen nehmen sie am Aequator allmälig eine schmutziggraue oder schwärzliche Färbung an. Namentlich sieht man häufig dunkele Streifen in radialer Richtung von dem schwarzen äusseren Rande der Berührungsfläche, der zugleich die Grenze des freien Theils der Eizellenmembran bildet, bis zum Rande der Bruchfläche sich hinziehen, was die Vorstellung von einem lebhaften centripetalen Zuge erwekt, mit welchem die Scheidewände der Abschnürung zustreben." It would appear from this, I think, that Remak located this phenomenon in the "Scheidewände" rather than elsewhere, and

* As is well known, Remak distinguished between what he calls "Einfurchung" and "Durchfurchung." The former is accomplished by an annular furrow, which advances only a certain distance toward the centre of the egg, and is accompanied by the involution of the "egg-cell membrane." From the floor of the furrow thus formed there proceeds, without (direct) participation on the part of the "egg-cell membrane," the formation of a new structure, — partition walls (Scheidewände), — which completes the separation of the halves of the segmenting egg. This last supplementary act is consequently called *Durch*furchung.

this view seems to be corroborated by the explanation (p. xxviii.) of the figure, viz.: "Fig. 2. *s*, Die Scheidewand; im oberen Theile des Eies zeigt sie graue radiale Streifen." Also in the explanation (p. xxix.) of Fig. 7 a (same plate): "*x*, einer an beiden Eihälften sichtbaren weissen Bruchfläche, an deren Peripherie die Scheidewand ähnliche radiäre Streifen wie bei Fig. 2. darbeitet." If the rays were thus superficial, as Remak implies, they could hardly be considered as belonging to a real protoplasmic aster.

It is quite another question whether Remak was right as to the location of the "Streifen," — whether they may not, after all, have belonged to the deep portions of the protoplasm.

An examination of the figures which O. Hertwig (**'77**, Taf. IV., V.) gives of the radiate appearances in different stages of the eggs of Rana temporaria furnishes nothing which would warrant us in referring the figures of Remak to like phenomena. It seems to me that the peculiarities which Remak has figured may perhaps be due to variations in the thickness of the [thin] pigment-lamella which, according to Hertwig (*op. cit.*, p. 49, foot-note), sinks down into the yolk at cleavage. Unfortunately Hertwig gives only a profile view (Taf. V. Fig. 6) of this lamella, from which it is not possible to determine whether its thickness is subject to alternations capable of producing, as suggested, this radiate effect.

Meissner (**'56**, pp. 374, 375), writing of Echinus, says: "Das Keimbläschen ist in zur Ausstossung reifen Eier bereits verschwunden; die Dotterkörnchen zeigen eine sehr deutliche radiäre Gruppirung um ein helles Centrum, welches sich als ein röthlicher, zähflüssiger Tropfen isoliren lässt. . . . . Der die Stelle eines Kerns vertretende röthliche zähflüssige Tropfen, der oben erwähnt wurde, theilt sich und der Dotter sondert sich in zwei Massen, deren jede sich um ein Centrum wieder radiär gruppirt."

The following year Gegenbaur (**'57**, p. 7, Fig. 3) described similar appearances during the first and second segmentations of the egg of Sagitta. A little while after the segmentation has been accomplished, the fine molecules of the yolk each time appear collected in a greater abundance around the nucleus, forming radial streaks (Streifen), which gradually disappear toward the periphery.

Further observations on the sperm-cells of Nematodes were made by Munk, Walter, and Claparède.

Munk (**'58**, pp. 382–391, Taf. XV. Figs. 11–14, 25, etc.) figures the structure in question for both parent and daughter cells. The "strahlige Anordnung" was observed in Ascaris mystax, A. marginata, and A. megalocephala (p. 382). In the formation of the four daughter cells, "The clear central nucleus of the radiate cell vanishes, and there appear nearer the periphery two new clear places, each of which becomes again the centre of a radial arrangement of the granules." Then follows the division of the cell. This process is then repeated on each of the new cells in like manner. When the four daughter cells have become free, the "strahlige Anordnung" of the granules soon disappears, and needle-shaped granules are no longer to be seen.

WALTER'S (**'58**, p. 493, Taf. XIX. Fig. 31. C. 6–8) observations relate to the sperm-cells of Oxyuris ornata, of which he says that, while the cells are growing, a granular mass is gradually deposited around the nucleus, which by and by assumes the well-known radial figure peculiar to some Nematodes.

CLAPARÈDE (**'59**, pp. 52, 60–63, Pl. V. Figs. 16, 17, Pl. VII. Figs. 3, 4) confirmed the observations of Reichert and Meissner so far as regards the occurrence of a radial arrangement of the granules in the sperm-producing cells of Ascaris mystax, etc. The nucleus entirely disappears, even beyond the possibility of being recalled by the use of acetic acid. Then the granules, hitherto irregularly distributed, move toward the periphery and arrange themselves in rays about a clear non-granular centre. In this stage the cells undergo a proliferation. The nucleus (i. e. the granular mass, which Claparède does not, however, confound with the clear nucleus, now disappeared) divides indifferently into two, three, or four parts. At first there are formed two or three clear spots at the centre of the granular mass; gradually these separate from each other, and around each of them the granules arrange themselves in rays. Thus are formed several masses with radial structure. The cell suffers constriction around each of these nuclei, and is finally divided into as many daughter cells as there are new nuclei. Claparède is probably wrong in considering these granular masses nuclei, for they must correspond to the asters which belong properly to the protoplasm surrounding the nucleus.

In the case of Phallusia mammillata, KOWALEVSKY (**'66^c**, p. 4, Taf. I. Figs. 2, 3) has given very clear drawings of optical sections of the cleavage spheres after the first and second segmentations. Concerning the radial phenomena, he says: "Die Dotterkörnchen der Furchungskugeln liegen strahlenförmig gegen den Kern."

LEUCKART (**'67–76**, p. 90) also alludes to the same phenomenon when he says of the first segmentation in Ascaris: "Die Dottermolecüle gruppiren sich um die aus einander rückenden Bläschen, wie um ihre Mittelpunkte."

While KUPFFER (**'70**, p. 128) corroborates for Ascidia canina the previous observations of Kowalevsky on the radial arrangement of the yolk granules about the nuclei of segmentation spheres, he places emphasis on the fact that it is not peculiar to segmentation spheres, since one finds it as much, if not more, pronounced in ovarian eggs after they have become altogether granular; "especially in the immediate periphery of the germinative vesicle the arrangement of the granules is at times so regular that one fancies he sees a crown of rods.

KOWALEVSKY (**'71**, Taf. IV. Figs. 26, 28, 29) has represented a delicate radial structure *in* what he considers the nuclei of the cleavage spheres in the case of Euaxes. That Kowalevsky considers this clear radially streaked protoplasm to be the nucleus, is sufficiently evident from his explanation (p. 63) of Fig. 26. I think it is quite certain, however, that the radiate structure is no part of the nucleus, but is homologous with the molecular asters of the yolk, as Bütschli (**'76**, p. 398) has already pointed out.

The studies of Schneider and other recent observers who have seen astral figures will be considered further on.

There have been observed numerous radial phenomena, which, at various times, appear in different parts of eggs and other cells. These observations, to most of which my attention has been directed by Bütschli (**'76**, pp. 387, 388), are, I believe, not all based on phenomena homologous with one another, to say nothing of their being identical with molecular asters. Many of them relate to the eggs of vertebrates, and the appearances have in such cases often been referred to the presence of pore-canals. In how far any of them may be referred to the same causes which induce the temporary radiate structures now generally known as asters, can perhaps be satisfactorily determined only by renewed studies. Without additional investigation it seems hardly justifiable to assume, with Bütschli, that all such phenomena are identical. Among the several cases which he cites, the observations of Eimer (**'72**[a], p. 219, Taf. XI. Fig. 3) exhibit as strong evidence as any that they are based on the study of asters; for the thick transparent membrane about the germinative vesicle is not very sharply limited from the yolk substance, the radiation about the vesicle is not fully restricted to this clear zone, and the early disappearance of the structure points to the unstable and temporary character of the phenomenon in this case. Still, I think it may be doubted if any of the cases cited below show very close relationship with the molecular asters, since the latter rapidly appear and disappear in advanced stages of the maturing egg, and during segmentation. Whereas the real asters seem to point to a fundamental rearrangement of already acquired substance, the appearances in most of the cases cited seem either to sustain important relations to the acquisition of new material, or to represent other permanent structural differentiations of the cell.

Reichert (**'56**, pp. 103–124, Taf. II., III.) has described at length a peculiar structure of the nutritive yolk in mature and fertilized eggs of the pike. At first sight the radiate structure in this case presents a striking resemblance to molecular stars, and more particularly to those modified spiral asters which have been traced in Limax during the formation of the polar globules. There seems, however, to be little ground for considering them in any way homologous. Reichert states that traces of the radiate structure are to be seen in fresh eggs, although much more distinctly when hardened in acid or alcohol. In sections of eggs thus treated, the striation may be seen with the unaided eye. The whole nutritive yolk is traversed by light and dark radiating streaks. These extend from the whole periphery, apparently converging at the centre of the yolk. This central region, or vertex (Scheitel-Region), is not spherical; its greatest extension corresponds with the sagittal plane of the embryo. The rays, rarely rectilinear, usually take a protracted S-shaped course. The streaking is somewhat finer at the posterior portion of the yolk than elsewhere, and is coarser in the middle part of each ray than at either end. The rays are due to fine canals which traverse the yolk from the periphery, where they begin with narrow openings, to near the centre. Instead, however, of terminating at the centre, they curve backward toward the surface. Each canal thus becomes continuous

with another radial tube of its own hemisphere, and the two describe a parabolic curve convex toward the centre of the yolk. The canals contain a liquid albuminous substance. In diameter they vary from 0.01‴ to 0.0025‴ (22.6 μ to 5.6 μ). They may subserve, according to Reichert, the purpose of an exchange of substance between the yolk and surrounding media, through a process of diffusion.

These canals continue to be traceable during the growth of the embryo, until the yolk is reduced to a very small mass.

Pflüger ('63, p. 79, Taf. V. Fig. 7) describes the "inner yolk" of the nearly mature egg of the cat as being often quite sharply limited from the "outer yolk," and still as presenting at the periphery a radial condition. One sees, he says, that at different, though not numerous places, sharply limited processes (which, I may parenthetically add, are very broad, and in no way recall the familiar aster) reach out from the inner yolk to the *zona pellucida*. "Man könnte dies," he continues, "auch so auffassen, dass man sagte, es bestände im Eie um das Keimbläschen eine Höhle, welche durch radiär verlaufende sich allmählich verjüngende Canäle mit der *zona pellucida* zu communiciren scheint."

The observations of Oellacher ('72, pp. 6–14, Figs. 3–10) on trout eggs led him to the conclusion that, while the egg is still in the ovary, the germinative vesicle approaches its surface, and that the thick radially striate membrane of the vesicle subsequently becomes evaginated through an opening at its most superficial point. The contents of the vesicle thus become eliminated from the egg, and the thick membrane is spread over a considerable portion of its surface as a thin veil (Schleierchen), which still continues to exhibit the radial (now palisade-like) structure. In the opinion of the observer the alternating dark and light striations are due to pore canals; the lumen appears dark (in a surface view as dark points), and the walls give rise to the light bands. Bütschli inclines to the opinion that in this case the so-called membrane of the vesicle is only a portion of the formative yolk which has become radially striate. But, to judge from Oellacher's Figure 6, the effect of this striating influence does not extend into the yolk deeper than the rather sharply limited substance called membrane, and its persistence throughout all the vicissitudes of an evagination and ultimate elimination from the yolk bespeak for it a mechanical condition which is hardly paralleled in the molecular asters.

In the eggs of certain snakes Eimer describes ('72[a], pp. 219, 220, 427, 428, Taf. XI. Fig. 3) the germinative vesicle as surrounded by an inordinately thick, very peculiar envelope (Hülle), "die aus sehr feinen Körnchen zusammengebacken scheint und sich durch eine schöne radiäre Streifung auszeichnet." In some places the rays are traceable into the surrounding yolk. In smaller eggs one finds only a thin membrane; in those that are larger this phenomenon has disappeared, and there remains simply the thin membrane. Eimer explains this appearance as due to pore canals. "Die radiäre Streifung ist wohl als der Ausdruck von *Poren*, von feinen Röhrchen zu erklären, welche die Hülle durchsetzen."

In addition to this circumvesicular striation Eimer ('72[a], p. 228, Taf. XI.

Figs. 8, 12, 14) describes radial structures in the cortical portion of the yolk, which are due, in his opinion, to the inward prolongation of the trumpet-shaped cells of the *granulosa.*

It is evidently to this latter structure that SCHULTZ ('**75**, pp. 577, 578, Taf. XXXIV. Figs. 8, 9) alludes when — comparing his own observations on the structure of the egg of Torpedo with those of Eimer — he says : "Notwithstanding a superficial agreement, a genetically determinable analogy is wanting, inasmuch as Eimer derives the protoplasmic streaks in the yolk of the reptilian egg from the penetration of the egg envelopes by the protoplasm of the cells of the *granulosa*, — a thing which must be absolutely rejected for the egg of Torpedo." The protoplasmic streaks in the egg of the Torpedo occur, according to Schultz, in the peripheral zone of the yolk as a series of wedge-shaped groups of fibres (Stränge) which consist of faintly granular protoplasm. The bases of the wedge-shaped groups are directed toward the periphery, and the radial fibres are connected with an irregular network which occupies the balance of the peripheral yolk.

In addition to these observations to which Bütschli has called attention, it may be observed that LEYDIG ('**57**, pp. 14, 346) has reported a structure similar to that of the so-called membrane of the germinative vesicle in the nucleus of certain large cells found in the "fat body" of Phryganea and other Arthropods, and does not hesitate to predict that this pore-canal structure may in the future be demonstrated in other cases.

In Fig. 194 (p. 362, *op. cit.*) Leydig has represented the secreting cells in the tip of the liver tube of Gammarus as conspicuously radiate in structure about the nucleus as a centre.

SEMPER ('**57**, p. 361, Taf. XVI. Fig. 3) figures large connective tissue cells from the stomach of Lymnæus in which the nuclei are surrounded by a narrow zone of finely granular substance, which is depicted as stretching out in irregular rays.

KÖLLIKER ('**57**, p. 92), moreover, has observed a radiate structure in the wall of the germinative vesicle in young eggs of Gadus lota, which he thinks may be due to pores. He adds : "Da nun *auch der Dotter*, so lange er noch feinkörnig ist, manchmal wie *äusserst fein radiär streifig erscheint*, so wird einem der Gedanke nahe gelegt, ob nicht vielleicht der ganze Stoffwechsel der Eizellen in bestimmten radiären Bahnen vor sich gehe u. s. w." Compare also Kölliker, '**63**, p. 17.

In the "Parenchymzellen" of Lampyris, MAX SCHULTZE ('**65**, Taf. V. Figs. 4, 5) has also figured radiate appearances, similar to those represented by Semper, in the immediate vicinity of the nucleus.

OELLACHER ('**72**^{b}, pp. 375, 376) has also observed, in the nutritive yolk of the trout's egg hardened in weak chromic acid, canals similar to those described by Reichert which open on the surface of the yolk beneath the vitelline membrane. The contents of the canals, of unknown composition, appear transparent and colorless. On the broken surface of eggs hardened in more concentrated acid ($\frac{1}{2}$ – 1%) a radial, striate texture is discernible. Both this and

the canals are doubtless due to peculiar structural conditions of the fresh yolk, though it is doubtful if they are identical.

Balbiani ('**73**, p. 77, Figs. 40, 79, 80) has seen a stellate arrangement of the protoplasm about the nucleus as a centre in the blastoderm cells of Epeira in a fresh condition.

Of interest in this connection are also the stellate differentiations in the protoplasm of certain Rhizopods.

Grenacher ('**69**, p. 292, Taf. XXIV. Fig. 1. *d*) describes for Acanthocystis viridis an irregular central space filled with an apparently watery fluid, and located in the centre of this a minute, pale corpuscle, from which radiate on all sides numerous likewise pale, fine filaments, which fully agree with the axial filaments of the pseudopodia. A direct continuation into the pseudopodia was not observed.

Greeff ('**71**, p. 6) confirms Grenacher's observation for A. viridis and other species, as also for Actinophrys Eichhornii, and finds the rays to be continuations of the axial filaments of the pseudopodia.

Schulze ('**74**[a], pp. 380–382, Taf. XXVI. Fig. 1) has also observed a similar structure in the case of Raphidiophrys pallida, and established beyond doubt that it is not with a *siliceous* skeleton that one has to do. Schulze's experiments, however, also show conclusively, if proof were at all needed, that the structure in question can have nothing in common with molecular asters, which are not destroyed, like the radial structures in Rhizopods, by acetic acid.

b. Quiescent Nuclei.

Many exceptions to the idea that the nucleus is a homogeneous compact body have been recorded by the earlier embryologists and histologists, but it is only within a comparatively short time that general assent has been given to the belief that it is often an extremely complicated structure.

Owing to their size, the nuclei of egg-cells have been much studied. In certain animals, especially the lower vertebrates, they have attracted attention from the great number, and often the peculiar arrangement, of their nucleoli; as, for example, in the case of Alytes and several fishes studied by Vogt ('**42**, pp. 1, 4, 15, Taf. I. Figs. 1, 2), in Cyprinus auratus according to Meckel von Hemsbach ('**52**, p. 421, Taf. XV. Fig. 1), in turtles as described by L. Agassiz ('**57**, pp. 475–479, Pl. VIII., IX.), and in Rana as more recently investigated by O. Hertwig ('**77**, p. 36, Taf. IV. Fig. 1).

The following authors, especially, have made interesting contributions to our knowledge of the structure of the nuclei of eggs not in process of division.

According to Eimer ('**72**[a], pp. 216–220), the germinative vesicle in reptilian eggs is of an exceedingly complex nature. The nucleoli are subject to the law of concentric arrangement which the author has elsewhere pointed out for the nuclei of other cells. This arrangement reaches a culmination in such stages as are represented by him in Taf. XII. Fig. 18. The germinative dots are also

of a vesicular nature. In Tropidonotus natrix the centre of each such vesicular space is occupied by a "Schrön's grain," and in this are embraced a number of fine granules (Keimpünktchen).

Kleinenberg ('72, p. 41, Taf. II. Fig. 12) was probably the first to call attention to a differentiation of the contents of the germinative vesicle into "a viscid, plasmoid, filamentous mass and a more fluid substance."

In his paper on Hydra, it is shown how, by a process of vacuolation, the contents of the germinative vesicle are separated into a continuous thin layer, lining the nuclear membrane, and a thicker mass concentrated about the germinative dot. The intervening space is filled by a fluid clear as water. The latter is traversed, however, by numerous delicate filaments, which connect the peripheral and central accumulations of granular protoplasm.

Since the appearance of this paper, the reticular nature of the germinative vesicle has often been observed; for example, by Flemming (**'75**, p. 100, Taf. I. Figs. 14–20) in fresh eggs of Unio; O. Hertwig (**'75**, pp. 351, 352, Taf. X. Fig. 1, Taf. XI. Fig. 9) in fresh eggs of Toxopneustes and in the eggs of the mouse; Ed. van Beneden (**'75**, p. 690, **'76**[a], p. 64, Fig. 9, **'76**[b], p. 170, Pl. XIII. Fig. 9) in the rabbit and Asteracanthion;* Bütschli (**'76**, p. 218, Taf. I. Figs. 6–8) in Nephelis; R. Hertwig (**'76**[a], p. 77, Taf. III. Figs. 8, 9) in the sea-urchin and the frog; Giard (**'77**[a], p. 720, **'77**[d], p. 434) in Echinus miliaris; Fol (**'77**[c], p. 440) in Asterias, Sagitta, etc.; Hoffmann (**'77**[a], p. 33) and Whitman (**'78**[a], pp. 13, 14, Pl. XIII. Fig. 61) in Clepsine; and Balfour (**'78**[b], pp. 412, 418, 437) in Scyllium.

Beside an intranuclear network of finely granular pale substance in the germinative vesicle of Unio and Anodonta, Flemming (**'75**, pp. 95–105) discovered that eggs which have attained a certain stage of development embrace in their nuclei not a single, but two apposed nucleoli. These differ in their reactions and in size. The one, which is called the principal nucleolus (Haupttheil), resists more the action of acetic acid, and is stained more deeply than any other part of the vesicle. The other, which is called the accessory part (Nebennucleolus), exhibits the same reaction as do large numbers of smaller nucleolar structures which are distributed through the network and which vary greatly in size. They are all more readily affected (swollen) by acetic acid, and are less intensely stained than the Haupttheil. The nuclear contents are still less stained, and the intranuclear cords become in stained objects invisible. The accessory nucleolus, at first absent, is, during early stages in the growth of the egg, smaller than the principal nucleolus, but ultimately exceeds the latter in size. Flemming is inclined to regard the accessory part as a *product* of the main nucleolus (constantes Quellungsproduct) but not as resulting from division. For this reason the multinucleolar condition of a nucleus may be regarded in a different light from that in which Auerbach (**'74**) has considered it.

Trinchese (**'76**) was the first, I believe, to call attention to the reticulum in the germinative vesicle of the (immature) human ovum. He characterizes

* This network is the nucleoplasm of Van Beneden.

it as a network of very delicate protoplasmic filaments composed of a homogeneous matrix and of granulations arranged in line. Within this network, which stretches through the whole cavity of the vesicle, the germinative dot is suspended, so that the filaments which, by their ramifications and anastomoses, form the network proceed from the dot. A similar reticulum is found in the case of the cat, the rabbit, and mollusks. In the latter instance, there are, beside the germinative dot, other smaller spherical corpuscles, one to seven in number, which are intensely stained in hæmatoxylin, or carmine. A short distance from the germinative dot is sometimes seen a pale transparent vesicle, which is not stained.

In a subsequent note Trinchese (**'77**) finds, in addition to the principal germinative dot and several accessory ones,* an irregular body, to which he gives the name "grumo" (clot). This presents prolongations which are continuous with the network filling the cavity of the germinative vesicle. The germinative dot and the grumo are both stained in hæmatoxylin; if subsequently submitted to ammoniacal carmine, the dot remains violet, but the grumo is stained red.

In his recent paper on the structure and development of the vertebrate ovary, BALFOUR (**'78^b**) has given considerable attention to the structure of the nuclei, especially those of developing ova, and the successive changes which they undergo, — changes that are not brought into connection with cell-division. The ovaries studied were those of selachians (Scyllium, Raja) and mammals (principally rabbits). During the conversion of primitive into permanent ova (Scyllium) the nuclei undergo certain alterations which are the same whether the primitive ova retain their individuality and all become permanent ova, or in groups suffer a confluence into polynuclear masses (syncytia), out of which result a diminished number of permanent ova.† The nuclei increase in size, and, in place of being granular, become delicate vesicles filled with a clear fluid, containing, close to one side, a granular mass, which stains very deeply with coloring reagents. The granular mass becomes more or less stellate, and finally assumes the form of a reticulum, with one or more highly refracting nucleoli at its nodal points (p. 416). Balfour thinks that the deeply staining granular mass constitutes a *part*, but not, as is maintained by Semper, the whole of the nucleus (p. 393). In addition to the ordinary mode of increase for nucleoli (viz. by division into two), there sometimes appears to be a production of numerous smaller nucleoli within a larger nucleolus, comparable with endogenous cell-formation. These nucleoli doubtless become free. Certain structures, variable in size, which are found in the yolk, are thought to be nucleoli thus set free (pp. 412, 413). The reticulum, which is conspicuous in the germinal vesicle of freshly formed ova, becomes granular and less distinct in older ones. The

* A misprint in the Jahresbericht of Hofmann and Schwalbe (Bd. VI., Anat., p. 15) makes Trinchese appear responsible for the existence of several "Keim*bläschen*."

† A portion of the nuclei in the latter case atrophy and become "pabulum for the remainder"; the metamorphosis of these is, of course, not embraced in what follows.

nucleoli (1 – 3) increase gradually in number as the vesicle grows older, and often lie in close proximity to the membrane.

Similar observations are made (pp. 425, 432) on the nuclei of mammalian ova. "The nucleus of the cells [germinal epithelium] loses its more or less distinct network, and becomes very granular, with a few specially large granules (nucleoli). The protoplasm around it becomes clear and abundant, — primitive ovum stage." "A segregation takes place in the contents of the nucleus within the membrane, and the granular contents pass to one side, where they form an irregular mass, while the remaining space within the membrane is perfectly clear. The granular mass gradually develops itself into a beautiful reticulum, with two or three highly refracting nucleoli, one of which eventually becomes the largest and forms the germinal spot *par excellence*." Klein's statement that the "nucleoli" are accumulations of fibres is too sweeping, according to Balfour, since nucleoli often exist in the absence of a network, and the latter is certainly wanting in primitive ova. The differences of opinion between Balfour and Klein are not very radical, however, for the former considers that both network and nucleoli are composed of the same material, — nuclear substance.

There are probably no tissues in which the structure of the nucleus has been more attentively studied than in the ganglionic cells of nerve centres. It is the magnitude often attained by them, as well as the lively controversy concerning the relation of the nerve fibres to them and their nuclei, which has tended to make the latter the objects of oft-repeated observations.

In 1846 the studies of Harless (**'46**, p. 287) on the ganglionic cells of the lobi optici in the torpedo pointed to the union of the nucleolus with one or two nerve fibres. After treatment with iodine the fibre (Nervenprimitivfaser) may be traced, says Harless, as a *light yellow streak*, through the more deeply colored ganglionic body, up to the nucleus of the inner cell [i. e. up to the nucleolus].

Similar conclusions were reached by Axmann (**'47**) and Lieberkühn (**'49**), whose results are cited by Stilling (**'56–'59**, pp. 807, 814, 821).* The last author, though unable to confirm these observations, maintains (**'56–'59**, pp. 783–787, 793–796, Taf. XXV.) that the parenchyma of cell, nucleus, and even nucleolus is traversed, without recognizable order, by numerous extremely fine tubes (Elementarröhrchen), which together form a dense network. Open as these studies of Stilling doubtless are to the severe criticisms which have been urged against them, they nevertheless may not be exclusively the result of a failure to distinguish between artificial and natural appearances. Recent studies make very probable the existence of a greater complication of nuclear structure than has hitherto been generally accepted, though not lending any direct support to Stilling's views of the *tubular* nature of the filaments he has depicted.

Wagener (**'57**) defends Lieberkühn from the criticisms of Stilling (**'56**), and adds evidence drawn from the study of nerve-cells in Hirudo, Limax, etc., to show the existence of the nucleolar fibre.

* See also Stilling, **'56**.

FROMMANN ('**65** and '**67**) was the first, according to Flemming ('**78**[b], pp. 350, 351), to demonstrate on *fresh* objects, in addition to such as had been hardened, the existence of branching filaments within the nucleus. Frommann does not, however, seem to have directly stated that these filaments by anastomosing completed a genuine network. I have not been able to consult the original papers by Frommann.

COURVOISIER ('**66**, pp. 24, 25) finds in nerve cells of the sympathetic system that "there emerge from the nucleolus, from the innermost centre of the cell, fibres which are in part coarse and in part almost invisibly fine. These are most easily recognized in clear nuclei, which they traverse in a radial direction, and thus adorn with a star-like figure." They are the "Wurzelfäden" of Courvoisier.

It should be added in this connection, that subsequently Courvoisier ('**68**, p. 134) "could not establish with certainty stellar figures in the nucleus — processes of the nucleolus" — in the case of the frog. Compare also pp. 142, 143 of the last-mentioned paper.

SCHWALBE ('**68**, p. 60) has often observed a radial arrangement of the substance of the nucleus [ganglionic cells] with the nucleolus as centre. The granules of the latter, however, do not afford confirmation of Frommann's view, which would make them to be the optical sections of nucleolar *fibres*. Vacuoles, although occurring in nerve cells of Arion (*loc. cit.*, Fig. 15), are not to be found in those of the vertebrates studied by Schwalbe (p. 63).

ARNDT ('**68**, pp. 473–492) recognizes no less than four kinds of "nucleolar filaments" in the cells of the cerebrum, but all are, in his opinion, to be otherwise explained than as structural filaments which traverse the substance of the nucleus. Either they are the optical expression of fissures in the nuclear substance, or really lie outside the nucleus, or are due to phenomena of refraction (p. 476).

After his discovery of a hollow sphere of granules surrounding at some distance the nucleolus in cells from the skin of the mole's muzzle,* EIMER ('**72**) extended his observations on the structure of the nucleus, and came to the conclusion that this "Körnchenkreis" was a very general feature of nuclei in the full vigor of life.

The clear area which Eimer described in the first-mentioned paper as immediately surrounding the nucleolus, he finds almost always present. That portion of the nucleus, however, which lies outside this "clear area," and from which it is separated by the "circle of granules," is not always dark, as reported in his first article, but may present the same appearance as the "clear area."

In his paper on the eggs of reptiles Eimer ('**72**[a], p. 236) finds further confirmation of this peculiarity of the nucleus in the follicular epithelium. He also gives figures of the division of the *nucleolus* (Taf. XII. Fig. 26. *a*) which strikingly recall certain phases of the process of division in the *nucleus* as at present understood.

In studies on nerve cells of the sympathetic system, and on the skin of Sala-

* Eimer, '**71**, p. 189, Taf. XVII. Fig. 8.

mandra, LANGERHANS (**'71**, p. 16, Fig. 6, **'73**[a], p. 750, Taf. XXXI. Fig. 11) confirms Eimer's observation. It may be added that EBERTH (**'63**, Taf. XIV. Fig. 2) long ago figured nuclei from the ephithelial lining of the triton's lung presenting similar features.

S. MAYER (**'72**, p. 812) says, "The substance of the nucleus [sympathetic nerve cell] is not homogeneous; there may be observed in the same fine filaments (Fäden) which arise from the nucleolus." Although unable to find a communication of the cell processes with the nucleus or nucleolus, he reports a little further on (p. 817) his conviction that, very often, in addition to the processes of relatively large calibre which arise from the cell-substance itself, still a second system of very fine filaments emerges from the cells which take their origin in the nucleus and nucleolus.

As part of a scheme of extensive, if not universal, applicability to the structure of protoplasmic bodies, HEITZMANN (**'73** and **'73**[a]) maintains for the nucleus a reticulation, which is continuous with a similar network of the surrounding cell-protoplasm. The reticulated structure, however, is an indication of a certain advance in the age of the protoplasmic body. "The originally quite homogeneous mass of protoplasm becomes differentiated at its periphery — with accompanying increase in circumference — into a network, while the centre — the nucleus — remains homogeneous. Then follows a differentiation in the central mass (nucleus) into a *Fachwerk*, and later into a network, so that here also compact, smaller centres remain as nucleoli."

Finally, the differentiation has taken place in the whole protoplasmic body. Thus the disappearance of the nucleus is followed by that of the nucleoli; the whole body is now only a network with coarser or finer nodal points, and this condition immediately precedes in tissues the formation of a "Grundsubstanz" (**'73**, pp. 155–158, and **'73**[a], pp. 46, 47).

These general conclusions are supported by observations of vacuolation in Amœba (**'73**, p. 101, **'73**[a], p. 42); by studies on blood corpuscles of Astacus* (**'73**, p. 105); on white blood corpuscles of man (**'73**, p. 107); on cartilage cells (**'73**, p. 142, and **'73**[a], p. 43); on ganglionic corpuscles of the brain and sympathetic centres (**'73**, p. 153); and on some other structures.

The nucleolus, the nucleus, the granules, and their filaments are the strictly living, contractile material, and this contains in its reticulations, and encases as a shell, a non-contractile fluid material, which, however, cannot be pure water.†

* In this case the corpuscles suffer a vacuolation resulting in a network which gives the protoplasmic reaction with chloride of gold, while the vacuoles remain colorless.

† The existence of an *extra*-nuclear network in the peripheral cells of the salivary glands of Blatta has been established by the researches of Kupffer (**'74**, pp. 78–81), who is not inclined to ascribe a passive importance, or even subordinate function, to the more pellucid, non-fluid "Grundsubstanz" through which the network runs. Compare also Kupffer's (**'75**) studies on the liver cells of the frog, etc.

An extranuclear reticulum of greater or less extent has been seen by many other observers in various tissues, but especially in nerve cells from the time of Stilling to Frommann, Trinchese, Ciaccio, and others.

Probably no single work within the past few years has furnished a greater impetus to the investigation of the structure of nuclei than the extensive and systematic observations published by AUERBACH (**'74**) in 1874. To avoid the possible influence of surrounding protoplasm on nuclear reactions, observations were conducted on nuclei mechanically separated from the cells, as well as on those left in their natural positions. Liver and other cells from the carp exhibit, besides 1–4 centrally located nucleoli, numerous exceedingly small and faint granulations (Zwischen-Körnchen) evenly distributed through the "Grundsubstanz," save that a clear area is left about each of the nucleoli and sometimes a corresponding area just within the nuclear membrane. In Auerbach's opinion, this appearance is due to the repulsive influence of the nucleoli and the nuclear membrane upon the "Zwischen-Körnchen," which are movably suspended in the soft matrix (Grundsubstanz). Eimer, however, has in Auerbach's opinion assumed too much in maintaining that this feature is general for fully active nuclei, and has reproduced appearances which only arise with the use of hardening reagents.

There is space here for only a brief statement of the action of reagents which Auerbach has described at considerable length. Water employed to *gradually* dilute the menstruum in which the nuclei are examined first causes a shrinking in the nucleus, accompanied by the expression of a hyaline fluid (Wasserschrumpfung). Accompanying this there may be an absorption of water from the more fluid portions of the nucleus by its denser constituents (innere Quellung), whereby an apparently homogeneous condition results. There is not, however, an actual melting together of the constituents. A more attenuated condition of the menstruum leads to a restorative swelling (Wiederaufquellung), which may, especially with pure distilled water, cause the nucleus to swell beyond its original proportions (Ueberaufquellung), but never destroys it.

Auerbach finds that solutions of common salt, bichromate of potash, acetic acid, and probably other substances, vary much in their effects upon nuclei, according to the degree of concentration employed. The hardening and especially the shrivelling effect is by no means always in direct proportion to the concentration of the reagent. A shrinking of the nucleus is caused by very dilute conditions of the reagent (extending from about 1% to 0.01%), and while even more dilute solutions (up to 0.001%) cause an "innere Quellung," it appears that there is often another and quite distinct series of concentrations (extending in common salt, e. g., from 1.5% to 14%) which produce essentially the same result. Solutions of sugar, whether concentrated or dilute, cause nuclei to swell. The shrinking influence of weak solutions of acetic acid and the swelling effect produced by solutions of sugar may therefore be made to counterbalance each other to a certain extent by a proper mixture of the two reagents, whereby a comparatively indifferent fluid may be produced. The action of neutral solutions of carmine is, aside from the staining, essentially like that of acetic acid.

These results, obtained by the treatment of nuclei mechanically freed from

the surrounding cell protoplasm, are corroborated by the deportment of nuclei which remain *intra*cellular, the only modifications being such as are naturally referable to the intervention of a protoplasmic layer surrounding the nucleus. Inasmuch as this protoplasm appropriates to itself a definite portion of the available substance of the solution, it is clear that it must exert a greater modifying influence when the solution is highly attenuated, than when it is more concentrated, — a conclusion which accords with the observed phenomena.

Of more importance for us than the confirmatory evidence of these views derived from the study of the nuclei of muscle cells, red blood corpuscles, etc., are conclusions reached in the second division of Auerbach's paper concerning the origin, increase, and vital properties of nucleoli.

Contrary to the opinion then commonly accepted, that the nucleus normally contains one or two, at most three or four nucleoli, Auerbach insists that the presence of more than four nucleoli is very often a typical condition, and that their number may amount to a hundred or more in extreme cases. This condition is as a rule the result of successive self-divisions of previously existing nucleoli, and the solitary nucleolus often has the same origin; the latter, however, may have an independent origin (Neubildung).

On the strength of his own observations in the case of frogs' eggs and the blastoderm of Musca vomitoria, supported by evidence drawn from previous observers, Auerbach concludes that in a certain first stage of embryonic development in the eggs of vertebrates, articulates, and worms the nuclei of the young cells are destitute of nucleoli. It may, moreover, be supposed, he says, that this enucleolar condition is for animals a constant element of a general law of development (p. 89).

In further elaboration of Reichert's law of *successive differentiation* the author concludes that in the beginning of organic life there is present only protoplasm, with or without yolk elements. In a second stage a differentiation of the protoplasm results in the formation of a homogeneous nucleus in the centre. A third stage is characterized by the appearance of a nucleolus in the centre, and a nuclear wall at the periphery of this nucleus. The last-mentioned structures are believed to arise directly from the protoplasmic body of the cell rather than from a differentiation of the nucleus, — the nucleolus to arise by the detachment of protoplasmic molecules from the protoplasm immediately surrounding the nucleus, which then migrate toward the centre of the soft nuclear mass (p. 84). Finally, a fourth stage is characterized by the appearance of intermediary spherules (Zwischenkügelchen) between nucleolus and nuclear wall. In case division ensues, the nuclear structures mentioned may be directly transmitted to the daughter nuclei, or it may be that the daughter nuclei are at first destitute of nucleoli.

Auerbach brings to the discussion of the relation between uninucleolar and multinucleolar nuclei an extensive series of observations upon various tissues of vertebrates, from which he feels justified in maintaining the existence of a parallelism between the Amniota and the Anamnia in so far as regards the predominance of multinucleolar nuclei in the higher representatives of each

phylum, and of paucinucleolar nuclei in the lower members of each group (viz. fishes and reptiles). What is thus established as a probable order of succession in a phylogenetic sense, is shown to be unquestionably true in the ontogeny of certain species, especially in the case of numerous tissues of Musca, where the growth of the larva is accompanied with a corresponding increase of nucleoli from one up to thirty or more. This increase of nucleoli is probably effected by successive divisions of the single nucleolus, and is accompanied by a gradual approximation of the nucleoli to the wall of the nucleus, perhaps as a result of an attractive influence exercised by the nuclear membrane. There are, he thinks, certain important reasons for considering the nucleoli to be of substantially the same nature as the cell protoplasm of young cells, viz.: —

1. Certain resemblances in their optical conditions;
2. The similarity of their micro-chemical reactions;
3. The tendency of large nucleoli to vacuolation;
4. The changes of form (amœboid) in the nucleoli; and especially,
5. The growth and self-division of the nucleoli.

As a natural deduction the nucleoli are considered as elementary organisms,— the equivalents of cytodes, or, if vacuolated, of nucleated cells,* —and the nucleus becomes from this point of view a breeding chamber (hohler Brutraum) in which the (endogenous) daughter cells (nucleoli) arise. Finally, attention is drawn to the possibility of identifying the liberation of such endogenous daughter cells with the histolytic processes described by Weismann.

In extension of Heitzmann's studies on the blood corpuscles of Astacus, Frommann ('**75**, pp. 289–294) has produced very interesting drawings of the nuclear and cellular reticulum met with in cells, which are probably also blood corpuscles of Astacus, though I believe no definite statement is made as to the source of the cells figured. Frommann was unable to verify Heitzmann's observation as to the method in which the vacuolated grains give rise to a closed system of network, especially since it could not with safety be denied that the apparently hyaline protoplasm took part in the formation of its filaments.

The study of thoracic ganglia in the crayfish yielded results confirmatory of statements made by him in a previous paper. The membrane of the nucleus is not of uniform thickness; there are, namely, within the nucleus granules and nucleoli which are closely apposed to, or fused with the membrane. The nucleoli are from three to ten in number, oval, round, or 3- to 4-angled, and may contain a more highly refringent grain in the centre. The angles are drawn out into coarse fibres, and there are, beside, finer threads connecting the nucleoli to surrounding granules, which in turn are joined to each other by short filaments. The nucleoli may be replaced by compact grains and fine granules. The filaments of the cell protoplasm unite with the nuclear membrane, with granules in it, or with nuclear granules lying inside the membrane,— in short, a direct connection between intra- and extra-nuclear network.

* This recalls the view which Vogt ('**42**) entertained concerning the nature of the "Keimflecke" of the germinative vesicle in fishes and amphibians.

Ed. van Beneden ('76[a], p. 65, Figs. 20, 21, '76[b], pp. 170, 171, Pl. XIII. Figs. 20, 21, '76[c-d], pp. 1188, 1193, Pl. I. Fig. 15, Pl. II. Figs. 19, 20) has described a nucleoplasmic network found both in the nucleus of the immense axial cell, and in that of the ectodermic cells of Dicyemidæ. It may traverse the nucleus in all parts, or may be more or less restricted. It is not found in young nuclei. The nuclear substance is stained rose-color in osmic acid followed by picro-carmine, and the nucleolus and nuclear membrane bright red, but the network is not stained.

Trinchese ('76[a], p. 175, Tav. II. Figs. 9, 29, and 32–38) confirms the existence of a previously announced ('76 and '76[b]) network in the substance of the cell and nucleus. In the present paper he shows its existence in epithelial cells, connective-tissue cells, and the cells of the albumen gland of Caliphylla mediterranea, and in the cornea of the frog. The points in which the filaments of the network meet present nodes or enlargements ; one of these nodes within the nucleus, somewhat larger than the others, is the nucleolus.

Studies on ganglionic cells of the retina of sheep, calves, etc. lead Schwalbe ('76) to the conclusion that the nucleus is differently organized at different stages of development. The smallest, and therefore youngest, nuclei (calf) are without the trace of nucleoli, and appear to consist of a uniformly distributed granular mass. This appearance is probably due to a netlike structure, but as yet there is no differentiation into contents and nuclear membrane. In larger nuclei 2 – 4 nucleoli are found within a clear mass, which is surrounded by a so-called nuclear membrane. Some of the nucleoli are intimately blended with the nuclear membrane, — appear as elevations upon its internal face. These become less conspicuous in larger nuclei, which usually contain but a single nucleolus, which is not attached to the membrane. In the sheep, ox, etc., this internal nucleolus exhibits thread-like prolongations which differ in extent and number in different cells. Schwalbe's interpretation of the observations is as follows.

The substance of which subsequently the nuclear membrane and the nucleoli consist — " nucleolar substance " — is at first uniformly distributed through the whole nucleus. This it fills, more or less completely according to the abundance of numerous small vacuoles distributed through it and containing another mass, " nuclear sap." In the growth of the nucleus there is an increase in the nuclear sap, without an essential increase of the nucleolar substance. The result is that the latter is sundered into various portions, one of which occupies the surface of the nucleus (nuclear membrane with its nucleolar elevations), while the other portions constitute the one or more contained nucleoli. Further increase in size induces a stretching of the membrane, which in turn causes its elevations to disappear. The whole process is a vacuolation. The nuclear sap, though probably a fluid, is by no means water, but contains albuminoids, salts in solution, and is at least of complicated chemical composition. The nuclear membrane and nucleoli, whether central or mural, consist of the same lustrous homogeneous substance.

Schwalbe agrees with Auerbach that in the growth of the nucleus an enu-

cleolar precedes a nucleolar condition, but does not believe, with the latter observer and Klebs (**'74**), that the nucleoli migrate from the cell protoplasm into the nucleus. Schwalbe agrees with Heitzmann so far as regards the existence of an intra-nuclear network, but not in its connection with an extra-nuclear (or cell) network.

The principal conclusions reached by R. HERTWIG (**'76**[a]) in his study of nuclei have been summarized by the author himself as follows : —

"1. The most important and characteristic part of the nucleus is the nuclear substance ('Kernsubstanz'), an albuminoid which, though possessing much in common with protoplasm, differs from the latter in numerous peculiarities.

"2. The nuclear substance is imbued, to a different extent in different nuclei, with a fluid, the nuclear sap ('Kernsaft').

"3. Primitive nuclei are simply naked masses of this 'nuclear substance.'

"4. From this primitive form of nucleus the remaining forms are derived by the following differentiations : —

a. The development of a nuclear membrane (nuclei of Infusoria).

b. The separation of the nuclear substance from the nuclear sap, whereby the latter

α. is irregularly distributed in the nucleus and forms numerous vacuoles, or

β. is disposed between the nuclear membrane and the nuclear substance, thereby inducing the formation of one or numerous nucleoli.

c. The invasion of the nuclear cavity by a nourishing protoplasmic reticulum, which traverses the pores of the membrane and crosses the space occupied by the nuclear sap."

"Nuclear substance" is, like protoplasm, capable of automatic motion, which may be either irregular (amœboid) or executed in a remarkably uniform manner, as during cell division ; but in other points — especially in its deportment with acids and with carmine and hæmatoxylin staining fluids — it shows constant differences from cell protoplasm. It is not maintained that "nuclear substance" is everywhere the same, any more than that cell protoplasm is identical in chemical composition in all cells. For the "nuclear sap" specific properties, which would allow it to be recognized as a thing *sui generis*, have not yet been established.

Often a portion of the nuclear substance remains in the periphery of the nucleus, thus forming a spherical mantle of homogeneous substance exhibiting the same chemical reactions as the nucleolus. This cortical layer of the nucleus (Kernrindenschicht) should not, in Hertwig's opinion, be confounded as has often been the case heretofore, with the nuclear membrane, which is a superimposed structure (Auflagerung), whether derived from the nucleus or from the protoplasm is unknown. The "Kernrindenschicht" is related to the "Kernmembran" much as the cortical layer of the protoplasm of the cell is related to the cell membrane, and like the last-mentioned structure the nuclear membrane is functionally a protective organ, since it cuts off the nucleus in its

quiescent state from the influence of changes in the surrounding protoplasm. The nuclear membrane and the "Protoplasmanetz" are functionally correlated, inasmuch as the nutrition of the nucleus, which is impeded by the former, is facilitated by the latter. This view agrees with the fact that both structures are limited to extensively differentiated nuclei.

Spengel ('76, p. 31, Taf. II. Figs. 33, 35) calls attention in his paper on the Urogenital System of Amphibia to a peculiar star-like figure which is occasionally found to take the place of a nucleus in cells from the testis of Cœcilia rostrata. The "Stäbchen" of this figure are stained in the hæmatoxylin as intensely as the nucleolus in other cases. Spengel thinks this appearance comparable with the process of nuclear division. Other structures, in form similar to Chinese characters, are often found in nearly all of certain groups or balls of testis cells.*

Flemming ('76) has communicated, in a paper devoted exclusively to the consideration of the nature of the nucleus, the results of studies undertaken with a view to determining whether the intranuclear network was present as a structure *intra vitam.* He made use of the urinary bladder of the salamander, and operated on animals that had been poisoned by the injection of a solution of curare, as well as on those which were not curarized. In both cases the tissues remained in the same condition.

With proper illumination it was possible to discern in endothelium, in muscular fibres, in cells of connective tissue (Bindesubstanzzellen), and in nerve-cells, this delicate intranuclear trestle-work; but only in a few cases can its connection with the nuclear wall be traced on all sides. The appearance is often so blurred that one might, without exercising great care, be led to a belief in the existence of only a granulation of the nucleus; the apparent granules, however, are the optical cross-sections of the fibres of the continuous network. Besides these apparent granules, there are present from one to three large nucleolar structures, "Hauptnucleolen," and often smaller nucleoli, "Nebennucleolen," which differ from optical sections of the network in which they lie by their greater size and different coloration or refractive power. In many of the nuclei nothing of all this can be seen, but the results obtained by the use of reagents are proof that, in these cases also, the same structure is not wanting.

By Hermann's method of analine staining still other structural differentiations than those already mentioned may be demonstrated, — "Analinflecke." These "Analinflecke" are not discrete corpuscles, but more intensely stained portions of the network; apparently they are only in part identical with the nucleoli before mentioned, as they are far more numerous than the latter.

In the discussion of the question, as to whether these appearances are due to

* Perhaps the latter are of the same nature as the remarkable modifications which the nucleus undergoes in certain cells of many invertebrates, especially Arthropods. Compare in this connection the following: H. Meckel ('46, pp. 33, 44, Taf. II. Figs. 26, 32, 33); Leuckart (Frey u. Leuckart, '47, p. 61, foot-note); Leydig ('57, p. 18, Fig. 8); Chun ('76, p. 47, Taf. I. Fig. 5); Helm ('76, pp. 444, 458, Figs. 1–13, 55, etc.); and P. Mayer ('78, p. 42, Taf. I. Figs. 6, 9, 10, 11).

coagulation, to artificial and post-mortem influences, or are present as structural features of living nuclei, Flemming offers the following reasons for regarding the former supposition as improbable : —

1. The regular form and arrangement of the network, which varies, it is true, with the use of different reagents, but within very narrow limits.

2. The constancy with which the nucleoli are found in the fibres (Balken) of the network.

3. The differentiations shown by the aniline staining to exist in the substance of the network, which would be homogeneous were the network only a result of coagulation.

"The network," Flemming therefore concludes, "is the expression of a given structural condition of the nucleus."

Although finding the network very distinct in fresh sections of hyaline cartilage from the salamander, and thus being able to confirm Heitzmann's discovery of the nuclear network in the cartilage of higher vertebrates, our author is unable to convince himself, from the study of epithelial cells, that the extra- and the intra-nuclear network are continuous, but states, on the contrary, that the network of the plasma and that of the nucleus deport themselves quite differently under the influence of the same chemical reagents. Flemming takes exception to some points in R. Hertwig's conception of the nucleus. He believes his present paper is sufficient proof that the intranuclear network is not limited to highly differentiated cells; and neither indorses its supposed nutritive function nor is able to find evidence that it comes from the protoplasm of the cell, — the less able since he finds no trace of pores in the nuclear membrane.

According to Lavdowsky (**'76**, p. 525, Taf. XXXV. Fig. 19. 1, 2, and 3), the ganglionic elements of the *ganglion spirale* often exhibit about the nucleolus the circle of granules described by Eimer.

Arndt (**'76**), the substance of whose paper I know only through Schwalbe's abstract in Hofmann u. Schwalbe's Jahresbericht, etc. (Bd. V., Anat., p. 25), considers the cell nucleus as composed of a homogeneous semi-solid "Grundsubstanz" and imbedded "Elementarkügelchen," each of the latter consisting of a capsule and a contained dark corpuscle. The "Grundsubstanz" is arranged throughout in the form of a network in whose interstices the "Elementarkügelchen" lie.

In the human *decidua serotina* examined in warm, fresh serum, one finds, according to Langhans (**'76**), that the nuclei are without trace of nucleoli or granules, — homogeneous, lustrous, and with even outline. With the use of reagents there promptly occurs (and in any event after the lapse of some time) a separation of its substance — either throughout the whole nucleus at once, or else beginning first at the periphery — into a highly refracting and a feebly refracting portion. The latter collects into numerous small, round, closely packed vacuoles. The former, the "septa," are at first of uniform thickness and very delicate. The vacuoles become larger and the septa break through at first near the periphery. Finally the whole nuclear substance ("Kernsubstanz") is

grouped into nuclear membrane, and 1–3 dark-outlined nucleoli. All the rest is a water-clear "Kernsaft." In the author's opinion the whole process is a "post-mortem disintegration."

Török ('77)* has observed in living dermal cells of embryos of Siredon that the "Dotterplättchen" suddenly change position, and that this locomotion is more precise in proportion as the nucleus is more sharply differentiated. No immediate relation exists between this phenomenon and changes of form on the part either of the cell or its nucleus, inasmuch as the latter are not accompanied by any rearrangement of the yolk elements. It is concluded from the study of osmic acid preparations that such Dotterplättchen movements result: (1.) Either in a group (or groups) of Dotterplättchen which melt together more or less completely and give rise to a network ball (kugelförmige Netzgebilde) which may occupy the same cell with one or several nuclei, or may take the place of a nucleus. (!) This reticular structure is succeeded by fine filaments having an irregular course, but the whole is not of the nature of a permanent differentiation, and eventually disappears. Or (2.) in a radiate arrangement of the Dotterplättchen about a centre of unknown significance. This is succeeded by a grouping of the same elements into a circle. With the widening of the circle there appear, as a differentiation of the central ends of the Dotterplättchen (possibly with the participation of the homogeneous protoplasm), radial rods lengthening ultimately into fibres with an increasing tendency to become curved (Stäbchen-Fäden). The Dotterplättchen disappear, and thus the whole cell loses its original character.

Both these structures are only transitional manifestations of cells in process of becoming tissues, and the "Stäbchen-Fäden" disappear earlier (in the embryonic life of the Siredon) than the "Netzgebilde."

It is perhaps attributable to his firm belief in the total disappearance of the germinative vesicle and in a like fate for the nucleus of embryonic cells, that Török failed to connect these remarkable changes with the process of cell division (to which they so clearly belong), rather than with that of the differentiation of cells into tissues. Yet it did not escape him that the disappearance of the nucleus was accompanied with similar transpositions of the "Dotterplättchen," only he maintains that in many cases they are independent of each other, so that, while in one cell new formative centres arise in the presence of a well-preserved nucleus, in another cell no trace of a nucleus of whatever kind is to be found.†

Of the changes in the nucleus, starting from the well-formed structure, Török says that a finely granular condition is followed by the appearance of coarser granules and an increasing transparency of the "Zwischensubstanz," accompanied by an increase of the mass of the nucleus. Then the coarse grains

* See also Török, '74.

† That new centres of attraction arise before a perceptible change takes place in the nucleus is conclusively shown in the present paper, and has also been pointed out by Strasburger ('76, p. 158) for Isoëtes.

are differentiated into rods, which are soon drawn together by a contraction of the Zwischensubstanz, lose their sharp outline, become irregularly curved, and so joined together that there results an indefinite network of coarser distinct, and of finer blurred fibres. This network is the typical form of the highest differentiation of the nucleus, which may vary in the fineness of its fibres and the closeness of its meshes in cells from different embryonic stages.

Often cells are to be found in which, by the side of one or two existing nuclei, two, three, or even four new nuclei may arise; and their development may be traced. (!) New nuclei may arise by a process of formative differentiation from the Dotterplättchen. Neither cell nor nucleus has always the same signification. A nucleus may later attain the dignity of a cell.

The studies of Török can hardly be considered as convincing proof that the prevailing histological notions are altogether out of joint. Few, I suspect, will be inclined to admit so prominent and active a *rôle* for the Dotterplättchen, even in those objects which form the basis of Török's studies, to say nothing of the cases where such "Formelemente" (e. g. Cucullanus) are wanting. It is not easy to understand why the sudden rearrangement of the yolk elements into a radial position is necessarily to be referred to the yolk particles themselves as the efficient living substance, rather than to the homogeneous matrix in which they lie. The proof of this assumed explanation appears to be entirely wanting.

That the changes described — certainly the "Stäbchen-Fäden" — are phases of nuclear division can hardly be doubted. Fig. 11 is particularly striking in comparison with the amphiasters of segmentation, and many of the other figures are readily comparable with stages of division which have been so exquisitely figured by Flemming in his last paper (**'78**^{b}).

In a recent communication, Eimer (**'77**) defends his previous papers from the charge of representing artificially produced conditions of the nucleus. The "clear area" receives the name of "hyaloid," and the "circle of granules" separating it from the peripheral portions of the nucleus is now called "shell of granules" (Körnchenschale), as expressing more accurately the spatial distribution of the granules. Stimulated to renewed studies of nuclei by the work of Heitzmann and Flemming, Eimer finds occasion to extend somewhat the nature of his earlier conclusions respecting the structure of nuclei, inasmuch as he finds that very generally the granules of his "Körnchenschale" are connected, each by a delicate radiating filament which traverses the "hyaloid," with the nucleolus, a clear indication of this arrangement having been already figured by him (**'73**, Taf. VIII. Fig. 82. *b*) in his Beroe studies. Further than this, Eimer corroborates the view that the nucleus consists of a hyaline "Grundsubstanz" traversed by protoplasmic filaments. Outside the "Körnchenschale" these filaments form a network of narrow meshes, which has usually been mistaken for a granular structure, but within that "shell" only the radial fibres are met with. The latter — and of course the "granules" — are nearly constant in number, so that about nine are in the field of vision

when the centre of the nucleolus is in focus. The granules of the "Körnchenschale" are separated from the surrounding network by a homogeneous space, so that a direct connection between the network and "Körnchenschale" cannot be made out except in rare cases, as when the granules are hardly distinguishable from those of the network. In such cases the granules of the "shell" are also directly united to each other by filaments.

An exceedingly fine protoplasmic network in the body of the cell (ciliate epithelium) is in connection on the one hand with the cilia, and on the other hand sometimes (gills of Axolotl) appears to be in continuation with the nuclear network (p. 116). The objects which Eimer has figured are principally ciliate epithelium from the mouth of Salamandra, the gills of Siredon and Anodonta, and certain ectoderm cells of Trachymedusæ. The first glance at some of the figures is enough to raise at once the question, if these appearances are not really due to phases of nuclear division; or, to be more exact, if the "Körnchen*kreis*" is not after all identical with the well-known "Kernplatte." The possibility of such an equivalency has not escaped Eimer himself (p. 111), but an attempt to explain *how* the "Körnchenschale" may be harmonized with the "Kernplatte," and the radial fibres with the spindle fibres, is not made, and there are many reasons to interfere with the establishment of such a comparison. If Eimer's descriptions permitted one to suppose that the granules of his "Körnchen*kreis*" were limited to a single plane, as that name naturally implies, an important objection would be cancelled; but they do not. If it were permitted to suppose — which it is not — that the central nucleolus did not occupy the same plane as the granules of the "Körnchenkreis," one might identify the radial fibres of Eimer's description with the "projected" spindle fibres, and his "nucleolus" with one of the poles of the spindle. Further, such an identification would necessarily compel its extension to those wheel-and-spoke figures, *several* of which are represented as occurring in a single nucleus (see Figs. 6, 9, and 10, *op. cit.*). I am unable to recall a parallel case of multiple *spindles* to place beside it. Perhaps the very recent studies of Flemming (**'78**[b]) will be sufficient to make more intelligible the relation of "Körnchenschale" and "Kernplatte."

In a note Flemming (**'77**) communicates the fact that he has observed the nucleoli and the nuclear network, previously described by him, in the *living and uninjured larvæ of salamanders*, so that the inference possibly to be drawn from the studies of Langhans, viz. that these phenomena are all post-mortem, is in no way justified. These conditions were observed on nuclei of connective tissue in the tail, the nuclei of nerve cells, nuclei of red blood corpuscles, etc. In the living condition, however, the structure is *pale*, and only to be seen with good light, a fact that may explain the account of Langhans. Moreover, the use of reagents may produce shrivelling and coagulation; nevertheless, the substantial identity of the fresh and hardened conditions cannot be called in question.

Stricker (**'77**) not only recognizes the existence of a reticulum in the nucleus, but has directly observed an amœboid motion of its filaments which he

regards as protoplasmic. He considers the cell nucleus as nothing more than an encapsuled portion of the active cell protoplasm, which may on occasion become free by a rupturing of the capsule into two or more pieces. He has observed, for instance, that in certain colorless blood corpuscles of Triton and the frog the nuclear envelope becomes broken through, and the intranuclear network and the cell protoplasm are thereby apparently in direct continuation. "The ruptured capsule rests on an amœboid mass (nuclear contents), like a snail shell on a crawling snail." In the case of naked, for the most part unchangeable, nuclei from the blood of the frog, Stricker adopts the term "Kernsubstanz" for the envelope and network of the nucleus (since they appear to be alike), and "Kernsaft" for the clearer mass which fills the interstices of the network. These naked nuclei are, however, genetically connected with uninuclear cells, inasmuch as the latter are seen to change in a manner which permits no other assumption than that the protoplasmic zone has withdrawn within the nuclear envelope. The retiring protrusions become smaller, and finally the nucleus appears naked. The protoplasmic filaments often break forth afresh, and the whole is again in motion. On such naked nuclei repeated *attempts* at division were observed. From all this it is to be concluded that the free nucleus with active (beweglich) internal network is only "ein abgekapselter Zellleib," and that the capsule is perforated or permeable.

In the blood of Triton and the frog there are still other elements, — finely granular, "sehr beweglichen," colorless blood corpuscles. In these the nuclei are not constant structures; they appear and disappear, and again are formed in the cell out of components of the cell body. The nuclear membrane is only a transitory formation, like the waves on water. While one portion of the nuclear membrane becomes invisible, a neighboring zone of the protoplasm is compacted (new nuclear membrane); the nucleus has thus become larger or smaller according to the position of this zone. The nuclei of these blood corpuscles, then, are not persistent formal elements.

The nuclei of the tissue cells, or fixed cells, are less changeable, but even here the network is sometimes (ciliate cells from the frog's palate) amœboid, and the nuclear membrane may change form, though it is not known to disappear.

"Als Merkmal der fixen Zelle mag der Kern noch von Bedeutung sein; als ein nothwendiges Merkmal der beweglichen Zelle kann ich die Existenz eines formell abgegrenzten Kerns nicht mehr anerkennen."

The optical effect of the reticulum in the living nucleus is quite other than that in the dead nucleus. In the latter one may speak of a "nuclear substance" and a "nuclear sap." In the living nucleus there must be, just as in the cell, an intranuclear fluid in the form of very small vacuoles; but it is not to be considered that the fibres of the net-work are bathed in the living cell by a "nuclear sap." The effect, on the contrary, is the same as though the reticulum were produced by a special arrangement of the living material, — as it were by an unequally distributed density of that substance.

Stricker considers as most important his observation of the disappearance and reappearance of the nucleus (active blood-corpuscles), and finds particular assur-

ance of the accuracy of his position in the fact that he has "*directly seen how a part of the nuclear membrane assumes the character of the rest of the cell body*, whereby the contents of the nucleus become one with this cell body." This would certainly be most important were it sufficiently established by Stricker's observations. It occurs to me, however, that the *direct optical properties* of the living protoplasm and nuclear membrane afford only one out of many criteria by which to judge of the identity of the two substances.

It were as competent to say of two fluids, that, because both are clear and of like refractive properties, consequently they are identical. It will be soon enough to accept Stricker's conclusions when it shall have been shown that no reagents are capable of disclosing a difference between the two substances of these cells during the stages of which he speaks.

ARNOLD (**'78**, p. 131, cf. also pp. 138, 139, and Taf. II. Fig. 1) affirms with some reservation the existence of deeply colored granules and filaments in the substance of the cell *and nucleus* of cartilage taken from animals, into whose blood indigo-carmine (sulphoindigotate of soda) had been infused.

KLEIN'S (**'78**) studies on the newt (Triton cristatus) were mostly conducted by the use of a 5 % solution of chromate of ammonia, followed by staining in carmine, picrocarmine, or hæmatoxylin. His results — so far as regards the structure of the nuclei — are very uniform for a variety of tissues, viz. epithelial cells of the stomach and the various components of the mesentery, surface endothelium, unstriped muscle fibres, connective-tissue corpuscles, — both migratory and fixed, — blood capillaries and lymphatics, and especially nucleated endothelial plates investing the nerve fibres. An extremely beautiful network of fibrils permeates uniformly the interior of all the epithelial nuclei, "*intranuclear network.*" The nuclear membrane is always well defined, and in some instances the network does not extend quite up to it, leaving an unoccupied zone. "But in all cases the network is in connection with what is known as the limiting membrane by numerous fibrils." How *these* fibrils differ from the fibrils of the network is not stated, nor why they are not an integral part of the network. The following description of the nuclear membrane seems to obliterate the distinction which was so clearly implied: "What usually appears as nuclear membrane is composed of an outer thicker portion, which is the limiting membrane proper, and — closely connected with it — of an inner more or less incomplete — probably because reticular — delicate layer, which is, properly speaking, a peripheral condensation of the intranuclear network, with which it is, of course, connected by longer or shorter threads. The clear space which may be observed in some instances between the 'membrane' of the nucleus and the intranuclear network is due . . . to a retraction of the latter from the former, and is a space, not between the two layers of the limiting membrane, but between the inner layer of this and the bulk of the intranuclear network."

The fibrils of the network are highly refractive, and vary in thickness, course, and arrangement. Almost always minute bright spots — more numerous in dense or shrunk networks — are to be seen; they are points of anastomo-

sis for the fibrils, or their optical cross-sections; moreover, some fibrils possess irregular thickenings. Nuclei examined in a perfectly fresh condition, with favorable light and powerful objectives (e. g. Hartnack, Imm. 10), show distinctly, although faintly, part of the intranuclear network. Klein disagrees with Flemming when the latter finds ground for believing in the existence of nucleoli fundamentally differing from the granules which are referable to thickenings in the network. At best, the so-called nucleoli are due to a shrivelling and intimate fusion of a part of the network, and are only transitory appearances. In the *fresh* condition it is demonstrable that the nucleoli are "accumulations of the fibrils of the network."

An intra*cellular* network is also conspicuous in most of the cells studied. In epithelial cells that have retained their cilia, the latter are seen to pass into the cell substance and identify themselves with the intracellular network, the fibrils of which in turn are in direct connection with the intranuclear network.

"In all muscle fibres the intranuclear fibrils may be traced to emerge as a bundle from the pole of the nucleus, and to become identified with the bundle of fibrils representing the core of the muscle-fibre itself." The nucleus possesses a small circular hole at each pole through which these fibrils emerge. Klein says that, after giving the point the greatest attention, he has been unable to find any evidence of a connection between the axis cylinder of nerve fibres and the intranuclear network; on the contrary, he is able in most instances to follow the axis cylinder along one side of the nucleus beyond the latter. This he thinks is the normal relation of the axis cylinder to the intranuclear network.

Flemming (**'78**[b]) publishes in a preliminary paper some of the results of studies conducted on the simple plan of choosing for examination such objects as permit a comparison of the living cells with stained preparations of the same. Numerous tissues, especially of the salamander, are made the objects of study, and the phenomena carefully reproduced in numerous figures.

The first of the two main divisions of the paper treats of the structure of the quiescent nucleus; the second, of cell division in growing and in inflamed tissues. The quiescent nucleus examined in the living condition exhibits a network which, however, varies in different cases, and is not so regular as often portrayed. The various appearances produced by the use of reagents — which vary in the reliability of the results produced — undoubtedly find their explanation in the existence of a corresponding structural differentiation in the living nucleus, but how far the intranuclear structures which appear after treatment with acids are identical with the living condition, cannot be so readily determined. Even with the best preservative reagents there is more or less distortion. The fine granulation of chromic acid preparations is held to be due to coagulation, as are possibly some of the smaller fibres of the network, but certainly not all. The effect of the chromates is especially untrustworthy.

The results touching the nature of the quiescent nucleus are summarized by the author himself much as in a previous paper (Flemming, **'78**). The quiescent nucleus consists of, —

(1.) A *mural layer* (Wandschicht), — the "nuclear membrane."

(2.) A substance distributed through its interior, connecting with the mural layer, and disposed in branching fibres, which do not exhibit any distinct regularity of arrangement (nuclear net, intranuclear network, or, better still, *intranuclear tressel*). The fibres (Balken) of this tressel present thickenings of variable form and number, — *reticular nodes* (Netzknoten).

(3.) Genuine *nucleoli*, which lie usually in the thicker, occasionally in the thinner, fibres of the network from which their substance differs. In the living condition the nucleoli — often several, but generally only one or two — are frequently not distinguishable.

(4.) A pale substance, which fills out the remaining intermediary space, and exhibits in the living condition no structure : — *Zwischensubstanz* of the nucleus.

Flemming especially insists upon the point that the *nucleoli* are not simply thickenings of the tressel-work, and defends his position by the results obtained in staining and decolorizing. It may be observed, however, that the process of decolorizing would necessarily take substantially the course indicated, even if the nucleoli were only concentrations of the tressel-work, since the removal of coloring matter must, *ceteris paribus*, permit the bulkier portions of the structure to remain longest in view. But this objection is anticipated by the author when he emphasizes the fact that the *Netzknoten* (inclusive of nucleoli) are often more intensely stained than their connecting fibres. "Die Netzknoten sind vielfach *absolut*, nicht bloss *relativ nach ihrer Grösse*, stärker gefärbt wie die übrigen Theile des Netzes." But the very fact that the Netzknoten stain intensely seems to me very unfavorable for the demonstration of a difference between the nucleoli and the tressel-work, for the Netzknoten are defined to be simply thickenings of the tressel-work.

Other conclusions from the results of staining are, that, since the nucleus is alone stained, or at least more deeply than the rest of the cell, it must be different from the remaining substance of the cell, — therefore different from "protoplasm." The staining of the nucleus affects all its parts, but the intermediary substance less than either the network, its nodes, or the mural layer.

In well-stained preparations the external limit of the nucleus is sharply marked, so that a connection of intra- and extra-nuclear fibres is doubtful. Flemming finds nothing to support the idea that the nucleus deports itself in staining like the plasm of colorless blood-corpuscles or young cells.

c. The Nucleus during Division.

Introductory.

As is well known, two fundamentally different views have been held about the condition of the nucleus at the time of cell division. According to one, the nucleus, on account of its dissolution and the distribution of its substance through the common protoplasm, disappears before

each division of the protoplasm of the cell, and the nuclei of the daughter cells arise as quite new structures; according to the other view, the nucleus of the parent cell gives origin directly to the new nuclei by a process of division, and the cases of supposed disappearance are to be explained as resulting simply from a temporary obscuration of the nucleus.

How firmly established the former view was with botanists in 1874, may be readily gathered from SACHS (Lehrbuch der Botanik, 4[te] Auflage, Leipzig, 1874, pp. 18, 19), who expresses his doubt about the *division* of the nucleus being in any way a general phenomenon.*

Doubtless an equally true reflection of the views prevailing at the same time among zoölogists is presented by GEGENBAUR (**'74**, p. 17) when he says: "The division is introduced by a division of the nucleus, and as a rule it can be established that the individual phases of the division of the nucleus precede the corresponding stages of division in the cell. In many cases, however, there appears to be a new formation of the nucleus."

From a comparison of these citations two conclusions can be drawn: first, that in both "kingdoms," as recently as 1874, *two radically different methods* of deportment were admitted for the nucleus; and secondly, that opinions were still at such variance as to allow very little room for a parallelism between plants and animals in regard to the persistence of the nucleus during cell division.

Aside from the disappearance of the old nucleus, it was the increase in the size of the new nuclei from very small beginnings, which seemed to entitle the idea of complete nuclear dissolution to consideration at the hands of zoölogists.

Of modes of cell formation other than by division, zoölogists very generally accepted as well grounded a process of budding, in which the division of the nucleus into numerous new nuclei was followed by a simultaneous constriction of the protoplasm into a corresponding number of parts (compare Meissner, **'54**[a], p. 262); but a so-called endogenous cell production — the equivalent of the "free cell formation" of the botanists — has not shared with zoölogists the same confidence.

In the earlier accounts of cell division, the formation of a partition (or, more commonly, the lengthening and constriction of the nucleus) was observed to accomplish its division. The presence of two approximated

* "Dass übrigens das von Hanstein (Sitzb. der niederrhein. Gesellsch., Bonn, 1870) beobachtete Verhalten der Kerne nicht ganz allgemein ist, zeigen schon die Theilungsvorgänge in den Antheridien der Charen u. s. w."

nuclei in a cell with undivided protoplasm has sometimes furnished the basis for an *inference* that an actual division had taken place. Yet the most accurate, connected, and careful of these observations failed to disclose what improved means of investigation have shown to be of very general occurrence.

One thing, however, especially in the case of segmentation, had been very often recognized in the best observations; namely, that the nucleus about to divide was a *homogeneous* body, exhibiting neither membrane nor nucleolus, and often that its outline became quite indistinct.

Among the papers which take notice of this interesting peculiarity are the following.

In 1846 the attention of Von Baer (**'46**, pp. 36, 37) was attracted by the appearance of a "langgezogener heller Schein" in the eggs of Echinus soon after fecundation, and also before each segmentation of the yolk. At p. 39 he describes more in detail the condition of the mature egg: "Im reifen Ei des Seeigels erkannte man an einer Stelle seiner Oberfläche einen hellen Kreis, der etwa ein Achtel vom Durchmesser des ganzen Eies hatte. . . . Dass es nicht ein Bläschen oder eine Zelle, sondern ein sehr weicher Körper ist, was äusserlich als heller Kreis erscheint, glaube ich nach vielfältigen Versuchen, die ich mit mechanischen Zertheilungen und einigen Reagentien anstellte, mit bestimmtheit erkannt zu haben, obgleich dieser Körper bald in seiner Metamorphose völlig durchsichtig wird."

Lovén (**'48**, p. 545), describing the development of Modiolaria and Cardium, says that "the nuclei of the cleavage spheres have no nucleoli, and behave under the compressorium in no way like vesicles or cells. They appear to be solid, but of quite limited consistence. Their periodical disappearance can hardly escape observation, but it is more difficult to make out how this happens."

As we have already seen, Warneck was certainly one of the first to point out the peculiar modifications which the nucleus suffers before its constriction and division. He was even impelled — probably in part from the small size of the new nuclei when they first became visible as distinctly outlined structures — to the conclusion that the nucleus underwent actual diminution of volume during each act of division, without, however, losing its identity.

J. Müller (**'52**[a], pp. 16, 17) speaks of "das Keimbläschen oder der *helle Kern*," and states that it contains no germinative dot; and in the communication published in his Archiv (**'52**, pp. 11, 19) he expresses even more clearly this peculiarity. "Das Keimbläschen im reifen Ei

von Entoconcha ist völlig hell und hat eine einfache nicht doppelte scharfe Contour. In seinem Innern sind keine Granula und nichts einem Keimfleck Aehnliches zu erkennen, es ist durch und durch so zähe, dass man an der Existenz einer Membran zweifeln könnte. . . . Das Keimbläschen . . . gleicht daher mehr dem, was Von Baer in den reiferen Eiern des Seeigels den Kern des Eies nennt."

LEYDIG (**'54**, p. 28) especially makes clear this feature, when, in describing the segmentation of the eggs of a Rotifer (Notommata), he speaks of "der homogene helle Kern des reifen Eies — das Keimbläschen — u. s. w.," and (p. 102) "das Keimbläschen im reifen Ei — welches übrigens keinen Keimfleck mehr hat, auch nicht ein Bläschen, sondern ein homogener zäher Körper ist."

METSCHNIKOFF (**'66**, pp. 410, 411) affirms that in Miastor, after the "*Keimfleck*" *has disappeared*, the germinative vesicle divides into two nuclei of equal size. For Aphis the absence of the germinative dot also characterizes the nucleus before division (p. 438). In Nemertes, also, a very brief notice of the mature egg is given by Metschnikoff (**'69**[a], p. 50, Taf. IX. B, Fig. 1, *vg.*), in which he mentions the germinative vesicle as being large and transparent, but of an irregular form.

ED. VAN BENEDEN (**'70**, p. 39), although he *infers* (p. 31) that the division of the nucleolus precedes the division of the nucleus, and this in turn precedes that of the "germinative cell" in the case of Distoma cygnoides, admits (p. 39) that in Udonella the nucleus, which the "germinative cell" embraces and which represents the germinative vesicle [first segmentation nucleus], no longer has a clearly recognizable contour; it still remains, however, as a paler spot. In the mammalian egg, too, the same peculiarity of the nuclei of cleavage spheres is to be inferred, inasmuch as, according to Van Beneden (p. 179), "La vésicule germinative se conduit dans l'œuf absolument comme les noyaux dans les sphères de segmentation"; and (p. 174), "Il est incontestable aussi qu' à certains moments la vésicule germinative devient très-peu distincte et qu'il souvent impossible de la distinguer."

The division of blastodermic cells in Tegnaria domestica has been described by BALBIANI (**'73**, pp. 51, 52, and Figs. 64–66), and the deportment of the nucleus observed. "On voit d'abord le nucléus, de pâle et circulaire qu'il était, prendre une forme allongée et devenir plus réfringent." It is only the internal substance of the nucleus which divides at first; the more elastic enveloping membrane continues to hold these halves together for some time (see his Figs. 65 and 66). This connecting band, which Balbiani thinks to be the nuclear membrane, is unquestion-

ably the band of interzonal filaments, but the author failed to discover that it was composed of filaments, and in general overlooked all the finer details of structure which the use of reagents would have made apparent.

But these are enough to show how frequently had been noticed a change in the appearance of the nucleus before segmentation. Aside, however, from this peculiarity and the almost concurrent testimony as to a *lengthening* of the nucleus by those who believed in its division, little advance was made in a knowledge of the nuclear changes.

a. Segmentation. — The peculiar metamorphoses which the substance of the nucleus undergoes in the formation of the spindle figure, and the accompanying changes which manifest themselves in the surrounding protoplasm of the yolk just prior to cleavage, like most discoveries, have been only gradually comprehended. Yet the advances in the intimate knowledge of these changes within the last half-decade seem marvellous.

In the light of recent studies on maturation and impregnation, we are now able to say that most of the earlier descriptions of the lengthening, constriction, and ultimate division of the nuclear structure of the egg just before the first segmentation rest upon the observation of something else than the *germinative vesicle.* In most cases it has been either the nucleus or the amphiaster of the *first cleavage sphere* that has been seen and wrongly considered as the dividing germinative vesicle. The class of observations in question, then, deals not with the phenomena of maturation, but with those of cell division, and is therefore properly considered in this connection. But where, on the other hand, observations have been less connected, and a division has been *inferred* from the presence and close approximation of two nuclear structures, it may be that in some cases the *pronuclei* have been mistaken for the resultants of division. Such is probably the case, to cite a single example, with the observations of Kölliker (**'43**, pp. 77, 78, Taf. VI. Figs. 5, 6) on Ascaris dentata.

Of those who have seen something more than an elongation of the nuclear structure, it seems that among zoölogists Ratzel was the earliest; but as his observations probably relate to the germinative vesicle rather than to the primary cleavage nucleus, they will be considered under the head of "Maturation."

Another observer, who has given evidence of having seen in animal cells the structure which is now generally known under the name of "nuclear spindle," is the Russian embryologist, Kowalevsky (**'71**, p. 13). As is well known, this naturalist early made use of sections in his embryological studies of invertebrates. The first unequivocal view of the

fibrous nature of the structure in question is due to this method of investigation. In tracing the development of the oligochætous worm Euaxes, it was discovered that the formation of new cells by segmentation was accompanied by a peculiar modification of the "nucleolus." This is described for the stage in which the embryo consists of only four cells as follows: "The section [Taf. IV. Fig. 24] passes through the two small spheres *e* and *c*, and one sees that from these two spheres there are beginning to be formed two new smaller ones, in the composition of which the halves of the nucleoli and a small portion of the whole segmentation sphere take part. The nucleolus does not appear in the sections as a vesicle in process of division, but exhibits, in the old as also in the newly forming cell, two granular accumulations, which are united to each other by means of fine, granular, but very evident protoplasmic (?) fibres [Stränge]."

There can be no doubt, on examination of the figure, that the so-called granular accumulations are identical with the lateral zones of thickenings, and that the Stränge uniting them are the interzonal filaments. The former are represented as lying in two parallel planes, appearing consequently in the form of two parallel straight rows of prominent granules; the latter, as faint lines of much smaller granules, which are parallel or slightly convergent toward the granular accumulations which pertain to the smaller cell. No curvature is shown in these fibres, nor is there any indication of their continuation beyond the two lateral zones. The latter are so far apart that the one belonging to the larger cell lies quite near the centre of the spherical mass of protoplasm which the author leaves one to infer is the *nucleus* of the larger cell, but which unquestionably is that portion of *yolk* protoplasm* which is destitute of coarse granules, and which is so often seen to present a radiate appearance.

Such were the shadowy glimpses that had been caught of the nucleus in its metamorphosis, when, about the beginning of 1874, there appeared, independently of each other, four articles upon representatives of three of the main groups of invertebrates, — cœlenterates, worms, and mollusks, — each of which devoted considerable attention to the changes in the nucleus before segmentation, and especially to the stellate figures which hitherto had failed to attract much attention or to elicit theoretical notions as to their significance.†

* Bütschli ('**76**, p. 398) has already called attention to this as being the first observation on the nuclear spindle, and has also pointed out the incompleteness of Kowalevsky's knowledge of this structure, and its relation to the nucleus.

† From this point forward the two phenomena, spindle and stellar figures, may be considered together as internal changes of the cell during division.

Not being able to gain access to SCHNEIDER'S paper (**'73**) on Mesostomum, I am indebted to the citation in Bütschli (**'76**, p. 399) for the synopsis which follows.

In the fecundated summer egg first the outline of the nucleus, which Schneider holds to be the original germinative vesicle, [apparently]* disappears, the nucleolus alone remaining visible. Acetic acid, however, brings out the much bent and folded outline of the nucleus. Finally the nucleolus disappears, and the whole nucleus is converted into a mass of finely curled fibres (Fäden), which become apparent [only] upon application of acetic acid. In place of these thin fibres thick cords (Stränge) finally appear, at first irregularly, afterwards arranged in a rosette which lies in a plane (equatorial) passing through the centre of the sphere. These cords appear to form the outline of a flat, much-indented vesicle; however, by more careful observation one becomes convinced that its contour is often interrupted at the inner angles of the folds.† The granules of the egg have become grouped in planes which intersect each other in a line perpendicular to the middle point of the equatorial plane. Little of this arrangement is to be seen on the fresh egg, but it becomes prominent on those treated with acetic acid. When the cleavage begins the cords have increased in number and have become so arranged that part are directed toward one pole, the rest toward the other pole. Finally, the egg is fully constricted, and the cords pass into the daughter cells. The rows of granules stretch out and may be followed from one cell into the other. Bütschli does not hesitate, after examining the figures, to identify the "cords" with the "Kernplatte" and its lateral halves. According to him the interzonal fibres are also indicated in the figure. The same method of nuclear increase was observed by Schneider in the case of germ-cells of spermatozoa and numerous other cells of Mesostomum, as well as in the eggs of Distomum cygnoides.‡

* P. S. — The words enclosed in brackets I have interpolated since consulting the original paper.

† This description recalls in a vivid manner the figures which Flemming has quite recently given of the nuclear metamorphosis of *tissue* cells in the case of the salamander.

‡ P. S. — Since writing the above, Schneider's paper (**'73**, pp. 113 – 118, Taf. V. Figs. 4. *b*, 5 – 8, 11) has been secured. Besides assenting to what Bütschli says respecting the identity of the "cords" and the lateral halves of the Kernplatte, there are one or two points to which I would call attention. The interzonal filaments are represented in Schneider's Fig. 5. *c* (Taf. V.) as having each three thickenings, which collectively form, in optical section, three parallel bands of thickenings lying between

From this it appears probable that Schneider observed the metamorphosis of the nucleus quite as accurately as either of the three remaining observers, but failed to discover that the radiation in the protoplasm was from two separate centres rather than from a continuous line.*

Fol has observed ('**73**, pp. 474–476, Taf. XXIV. Figs. 2, 11) that in the fresh-laid, fecundated eggs of Geryonia the nucleus is unlike the germinative vesicle of the unfecundated eggs. It appears like a vacuole on account of the refractive power of its substance being less than that of the surrounding protoplasm. It is possible to distinguish a membrane (eigene Wandungen) around this vacuole only after treatment with acetic acid, and then with little distinctness. From this, and its size, Fol concludes it cannot be identified with the germinative vesicle of the unfecundated egg, which contains a vesicular germinative dot, but he retains for it, nevertheless, the designation of "germinative vesicle." Just before the first segmentation this germinative vesicle [primary cleavage nucleus], has a more confused look and undergoes many changes of form.

the much more conspicuous groups of "cords." The middle one of these three bands falls in the plane of cell division, and apparently corresponds to the cell plate of Strasburger.

The figures presented by Schneider recall even more forcibly than the text the similarity already alluded to, which exists between his observations and those of Flemming on certain tissue cells. Another point is the discovery, in the formation of spermatozoa, of cells "in der Viertheilung" (p. 117 and Fig. 8. *i*), presenting conditions parallel to those pointed out by Russow and Strasburger for pollen cells.

The opinion expressed above, that Schneider failed to recognize the radial structure as centring in two points, is possibly not quite just, for he says (p. 114, *l. c.*): "Die *polare* Anordnung der Körnchen findet man bekanntlich auch beim Furchungsprocess der Ascidien und Seeigel." Schneider (*l. c.*, p. 138) calls attention in the explanations of figures to the fact that the polar arrangement of the granules is insufficiently brought out in Fig. 5. *b*. Finally, this metamorphosis, which most likely occurs in those cases where the nucleus appears to vanish, is not the only method of cell division. Two methods must be recognized: one in which the nucleus undergoes the indicated metamorphosis; the other in which the nucleus retains its form (p. 115).

* P. S. — Compare this description with the pinnate arrangement of the astral figures recently described by Fol ('**79**, p. 167) for Toxopneustes.

Fol seems to have remained for some time ignorant of the discoveries of Schneider, as he makes, I believe, no mention of the latter's work except in the paper last cited, and there (*l. c.*, p. 207) states that he knows of Schneider's work only through Bütschli's citation. Since Fol appears to be in doubt as to whether Schneider's paper was published in the same year as his own paper on Geryonia, or the year following, it seems to be but a matter of justice to emphasize the fact that Schneider's paper was published in April, 1873, therefore about seven months *before* the paper in which Fol recorded his observations in the case of Geryonia.

It soon disappears entirely, but immediate treatment with acetic acid brings again to view what remains of it, — only a trace (Andeutung) of the former nucleus, — and at the same time two accumulations of protoplasm, whose closely massed granules assume the form of two regular stellate figures, one on either side of the remnant of the vesicle. The rays of these stars are formed by the granules, which are arranged in straight lines. Many such lines stretch from one star-centre to the other in an arch, thus embracing the remnant of the "germinative vesicle." A little later the acid fails to disclose a trace of the nucleus, but the stellate figures are unchanged save that they are farther apart.* By the segmentation of the yolk these *centres of attraction* become more and more separated, and there now appear in each of them one, two, three, up to eight or ten small vacuoles, which ultimately melt together and become so rounded as to present exactly the same appearance as the undivided "germinative vesicle."

Such is the formation of the new nuclei. These, with similar observations on mollusks, worms, etc., lead Fol (p. 487) "to accept in full Sachs's theory of segmentation by Anziehungs-Mittelpuncte." "At segmentation the 'germinative vesicle' every time disappears, and in its place there arise in the protoplasm two centres of attraction, in which latter the new nuclei appear" (p. 486).

Bütschli's (**'73**[a], pp. 101–104, Taf. XXVI. Figs. 61[c], 61[d]) studies were made on the egg of Rhabditis dolichura, Schneider, without the use of reagents. They really cover earlier stages in the ontogeny than the observations of Fol, inasmuch as Bütschli observed the approach and contact of the two structures which we now know to be the pronuclei. When the latter have reached the centre of the egg, they appear almost as though melted together, and the yolk granules become suddenly grouped radially to the body thus formed. The latter lengthens in the direction of the long axis of the egg, and assumes a lemon shape. After some time one observes at either pole of this figure a knoblike protuber-

* When Auerbach (**'74**, p. 254) intimates that Fol's account is not quite clear, one has no opportunity to object; but when he makes Fol responsible for the [implied ?] statement that the nuclei of the first segmentation spheres are produced by a *division* of the germinative vesicle, and that the radial figures appear for the first time in the stages preparatory to the *second* segmentation, then it must be objected that the less plausible of two explanations is the one put forward. At least, I see no reason why Fol may not have called attention to a figure (Fig. 2) representing a *corresponding* stage in a *later* segmentation (just as Auerbach himself, p. 225, has done) to illustrate the phenomena of the *first* segmentation, without involving himself in the inconsistency with which he has been charged.

ance, which increases in size, and about which a stellate circle is formed in the yolk. The knobs continue to separate, while the connecting portion becomes reduced in thickness to a mere thread. This thread of connection remains some time, but finally during segmentation its halves are slowly contracted toward the nuclei to form, close to them, a new knoblike swelling. The radiate figures in the yolk now become less distinct; the outlines of the nuclei more definite. During the whole process of division the contour is confused, and, in addition to the radial figures of the yolk at the poles of the lemon-shaped nucleus, Bütschli seemed to see raylike processes stretch out from the nucleus into the substance of the yolk, which served to strengthen his conviction that the nucleus possesses at times a considerable degree of mobility. Whether, however, this phenomenon has anything to do with the radial arrangement of the yolk granules, he did not venture to decide.

The failure to see arched rays joining the two centres of attraction may readily be understood when it is remembered that his studies were made exclusively on living eggs.

The subsequent segmentations presented essentially the same phenomena. There is no such thing in his opinion as a disappearance of the nucleus, although before the division it becomes quite indistinct, a fact which he is inclined to connect with its mobility.

The conclusion seems to me unavoidable that the knoblike swellings at the poles of the lemon-shaped nucleus correspond to the centres of the stellate figures which Fol saw, and are by Bütschli connected too intimately (as parts of the lemon-shaped figure) with the nucleus. The knoblike swellings which are formed at the close of the segmentation out of the contracting thread are really the new nuclei, for which the centres of the stellate figures were mistaken. The radiate structure was also observed (p. 35) by Bütschli in the formation of the sperm cells.

In the study of a much less favorable object, Anodonta, Flemming (**'74**, pp. 286–290, Taf. XVI. Figs. 22–29) was also fortunate enough to see some of the phases already noticed by Fol and Bütschli. He found that many of the segmentation spheres under gentle pressure presented near their centres one or two clear spots without granules, and stretching out from these toward the periphery in an almost strictly radial direction rays of clear protoplasm (körnerloser Substanz), so that the granules which lay between these rays were likewise arranged in diverging lines. Subsequent to this condition followed a stage in which two nuclei were found in the undivided cleavage spheres. In no case were radial structure and nucleus found to be present at the same time.

Flemming erroneously concluded that the centres of the peculiar radial arrangements of the cell protoplasm were probably to be considered as formative centres (Bildungscentren) for the new nuclei, and asserts that the segmentation cells of the Anodonta germ actually pass through stages in which they are *without nuclei.**

While Fol and Bütschli agree in the interpretation of the radiate phenomenon as being the result of an *attractive force*, they are diametrically opposed on the old and cardinal question of the persistence or disappearance of the nucleus. While Schneider and Bütschli are in agreement on this point, Flemming's testimony is all in favor of the non-persistence of the nucleus. The last-mentioned observer justly makes prominent the fact, that the arrangement of the yolk granules is dependent on the condition of the clear protoplasm, — the star proper.

Since the appearance of these four papers much attention has been given to the phenomena which they discuss.

Whitman (**'78**[a], p. 16) cites METSCHNIKOFF (**'74**) as having seen and described the radiate structure in Geryonia (p. 19) and Polyxenia. As regards the entoderm cells of Geryonia (Taf. II. Fig. 7. B), I doubt if the structure has anything to do with the molecular asters developed at the time of segmentation. Metschnikoff himself speaks of these irregular fleecy-looking stretches of protoplasm as the "die bekannten Protoplasmaausläufer," which would hardly be the expression to be used of molecular asters. It is in reality a permanent phenomenon, (if one may speak of anything as permanent in a growing organism,) which is most prominent in the *least* active period of the individual cell's existence. In the segmentation spheres of Polyxenia before the differentiation of ectoderm and entoderm (Taf. III. Fig. 3), it may reasonably be claimed that the rays figured are due to the same influence as those which induce the molecular stars; but the figure, after all, is hardly more suggestive of the real aster with its multitudinous rays than are Grube's drawings in the case of Clepsine. I do not find that Metschnikoff makes any explanation of this figure in the text.

* The radical tone of this statement is considerably modified by the very restrictive definition which the author formulates for "nucleus." "Der Name Kern knüpft sich für uns einstweilen an bestimmte Merkmale: eine Membran oder eine scharfe Absetzung nach Aussen, einen von der Umgebung verschiedenen Inhalt und meistens einen Kernkörper." "Die Substanz des nicht mehr sichtbaren Nucleus wird jedenfalls in irgend einer Form in den Zellen noch vorhanden, vielleicht sogar localisirt sein; aber wer sie in diesem Zustand Kern nennen wollte, der würde mit gleichem Recht die Auflösung eines Kochsalzkrystalles als einen Krystall bezeichnen können." (!)

Salensky ('**74**^{a}, p. 332) mentions the presence of "ein kugelförmiges Klümpchen, welches aus den feinsten Körnchen bestand" within the germ cell of Amphilina eggs, and is inclined, on the strength of Schneider's ('**73**) discovery, to consider it as the altered germinative vesicle.

Schenk ('**74**, pp. 294–297, Fig. 8) describes the appearance of a clear portion in the middle of the yolk of Serpula, — after the egg has exhibited contractile phenomena and has eliminated the germinative dot, — which neither occupies the position nor possesses the definite contour of the vanished germinative vesicle. On the contrary, in a radial direction it loses itself in the surrounding protoplasm. This nucleus is not an isolated structure, nor does it differ essentially from the nature of the yolk, save that the yolk granules are there less abundant. Its increase accompanies the gradual disappearance of the space at one time existing between the yolk and its membrane. Sometimes this first nucleus with its radial streaks appears divided into two parts; this, however, usually occurs only just before the first segmentation.

Similar stellate nuclei were also seen in vertebrates' eggs. After segmentation it is often seen that one of the resultant spheres contains the whole nucleus, while the other only subsequently acquires one, which is formed just as was the nucleus of the first segmentation sphere. Schenk concludes by saying that one sees from this that the nucleus is produced by "a want of uniformity in the distribution of the granular mass," and that the nucleus is to be considered as a central part of the protoplasm, from which it is derived and with which it is intimately united. Subsequently Schenk ('**76**, Figs. 2 and 4) saw stellate figures in the eggs of Echinus after fecundation, and when the embryo consisted of four segmentation spheres.

The careful studies of Auerbach ('**74**, pp. 217–262) on the eggs of Ascaris nigrovenosa and Strongylus auricularis were especially trustworthy on account of their being continuous observations on living eggs, in which, however, a compressorium was employed.

After the complete union, near the centre of the egg, of two nuclear structures, which we now know to be like those seen by Bütschli, — the pronuclei, — Auerbach's observations are to the effect that the resultant structure becomes elongated in the direction of the long axis of the egg, and also suffers a reduction of volume. This continues till the structure becomes a very narrow stripe with parallel edges and pointed ends; then it looks like an exceedingly thin fissure in the protoplasm, and finally disappears; yet not absolutely without trace, for during this change the protoplasm surrounding it has become free from granules. This clear

portion of protoplasm has the form of a dumb-bell, the rodlike middle portion being the part which contains the "fissure" before its disappearance. From each of the spheres of the dumb-bell stretch out on all sides rays of clear protoplasm, between which rows or wedge-shaped masses of granular yolk are embraced. The rays are usually straight, sometimes slightly bent with the concavity directed toward the centre of the egg, and give the figure the appearance of two pale suns united by a long middle piece. The lengthening of the figure continues till the middle piece is more than half the length of the egg.

The formation of the dumb-bell figure begins with the radial arrangement of the yolk granules about the tips of the broadly spindle-shaped nucleus, i. e. only when the latter begins to lengthen. The pale rays intervening between the rows of granules become more conspicuous, and their bases unite to form the head of the dumb-bell, while the "middle piece" is forming.

These phenomena Auerbach explains as follows. The nucleus perishes, and during the lengthening of the nuclear cavity the sap which fills it penetrates between the molecules of the neighboring protoplasm, forcing the yolk granules out of it. The rays about the tips of the nucleus are the (physical) expression of the courses along which the fine streams of nuclear sap penetrate the protoplasm. The distribution takes place from these tips for two reasons: because the tip of the nuclear cavity presents a greater amount of surface, as compared with the contents, than any other portion, — hence the point of least resistance; and because the sap, on account of the lengthening of the nucleus, is in motion toward these two points. Subsequently, the resistance of the lateral walls of the nuclear cavity is so far overcome as to cause the recession of the granules from a thin layer of the adjacent yolk substance. The nucleus, however, is not the active element. The protoplasm acts on the passive nuclear sap by changing the form of the nuclear cavity, and by imbibing the sap. In place of a nucleus there is now in the yolk a peculiarly shaped territory free from granules, in which all the substance of the nucleus is dissolved, — the *karyolytic figure.*

The formation of this figure is followed by the segmentation of the yolk. The latter is accomplished by a furrow, which, advancing from one side only, passes through the yolk, or possibly sometimes by an annular constriction. Soon after the beginning of the segmentation there appears a vacuole at each of two corresponding points in the stem of the karyolytic figure near the cleavage plane. These are at first small, being irregularly and indistinctly outlined. They increase and

gain circular outlines while they migrate toward the poles of the figure. They are the new nuclei. They reach or pass the centre of the new cleavage spheres, but do not reach the swollen end of the karyolytic figure. The latter meantime gradually disappears. First the stem becomes slimmer; the rays become shorter, and then disappear; the centre of the sun becomes flattened to the form of a disk or a meniscus lens concave toward the cleavage plane; the stem disappears; the meniscus becomes thinner, and also disappears. According to Auerbach, the new nuclei are formed by the re-collection of the diffused nuclear sap into a single drop for each sphere; but inasmuch as each of these is larger than the half of the old nucleus, additional nuclear sap must have been extracted from the protoplasm.

It did not fail to impress Auerbach as peculiar that the formation of the new nuclei should be accompanied by their motion in substantially the same direction as that which prevails during the dissolution of the old nucleus, instead of the opposite direction; but it does not seem to have caused him any misgivings as to the accuracy of his theoretical propositions.

With Ascaris and Strongylus during segmentation the nuclei never acquire a membrane, and for this reason the membrane, when it does exist, must be considered as a secondary structure produced by a condensation of the layer of *yolk protoplasm* immediately enveloping the nuclear fluid. The nucleoli arise in the nucleus after it has come to rest, not before.* In regard to the exact manner of their origin, Auerbach in so far modifies the opinion held in the first part of his paper as to admit that they are not necessarily portions of the surrounding protoplasm which are subsequently detached and set free in the fluid of the nucleus, but that molecules of protoplasm may have been detached with the formation of the nuclear fluid, and have remained distributed through it till they at length became visible by becoming grouped into the observed nucleoli. The author is also less confident that a multinucleolar condition always arises by the repeated division of a single original nucleolus.

This method of nuclear increase, to use Auerbach's own words, "entspricht in der Hauptsache der einerseits von Reichert, andererseits von den neueren Phytologen aufgestellten Lehre. Aber es ist ausgezeichnet dadurch, dass die Substanz des aufgelösten alten Kerns nicht in dem ganzen Zellenleibe sich vertheilt, sondern in einem beschränkten inneren, eigenthümlich gestalteten, doppelt gegliederten und durch strahlige Fort-

* In the case of the pronuclei, it will be seen that Auerbach says the nucleoli arise *before* the former execute their migratory motion.

sätze erweiterten Bereiche, und zwar unter Verdrängung aller gröberen Körnchen aus diesem Bereiche." In his opinion, the old nucleus suffers complete morphological ruin.

This method of increase of nuclei Auerbach designates as "palingenetische Kernvermehrung," in opposition to that where no dissolution, but a direct division, of the nucleus takes place.

The central area of the stellate figures in the case of Limax presents objections to some of the views which Auerbach entertains. The fluidity of the nuclear sap should reduce the refractive power of this portion of the protoplasm; as a matter of fact the refractive power increases toward the centre of this area. The stellate centres are often at considerable distance from the waning nuclear structure, and yet the side of the aster toward the nucleus shows no differentiation corresponding to the supposed flow of nuclear sap. I have every reason to believe, however, that in Limax the central area of the stellate figure is, in the words of Auerbach, "not a nucleus, also not the formative centre of a nucleus, that it in fact does not even indicate the place at which the new nucleus appears, and that the latter, even in its migration, does not advance into [the centre of] the body of the sun."

Lankester (**'75**, pp. 39, 40) asserts that, in the case of Cephalopods, the cap of formative matter segregated to the smaller pole of the egg "presents no nucleus, persistently, though a nucleus *may* appear in it at the first." "I have most fully satisfied myself," he continues, "that temporarily many of the segmentation products are devoid of nucleus." The cells which result from the segmentation of the cap of formative matter Lankester calls "klastoplasts." Before the superficial extension of this cap of klastoplasts has begun, there appear in a deeper stratum of the yolk pellucid nuclei, at first arranged in a circle around the cap. These are called "autoplasts." They are of the same nature as the nuclei of cleavage segments. "I believe in the eggs of Loligo there may be, according to season, an increase of these nuclei, or, on the other hand, of these bodies, they being reciprocally vicarious within small limits." No area becomes segmented around the autoplasts; "they commence as minute points, gradually increasing in size, like other free-formed nuclei."

In his preliminary account of the development of Pteropods, Fol (**'75**, p. 196, also **'75**^{a}, p. 198) says that before each segmentation the nucleus disappears, to be replaced by two molecular stars which arise in its interior. The centre of each may be considered a centre of attraction: all the vitelline substance yields to this attraction. After the cleavage a nucleus reappears at the centre (*au milieu*) of each star.

Bütschli (**'75**, pp. 210 – 213) is impelled in his studies on Nematodes and snails to admit his former notion (that the nucleus simply lengthens and divides) to be untenable; but is not willing to follow Auerbach in concluding that the nuclear substance is distributed through the protoplasm. As regards the nuclei, they are, however, as Auerbach maintained, *new structures.* The most interesting part of Bütschli's discovery is the spindle-shaped body (p. 208) an account of which is given in another connection (see p. 536). Of the spindle which occupies the place of the primary cleavage nucleus after the latter has assumed an unrecognizable state, Bütschli says (pp. 211, 212) it greatly resembles the spindle just described. In the earliest stages of its visibility there lies a dark lustrous granule in each fibre at the equator of the body, so that in a view upon the end of the spindle the granules together form a circle. Changes, similar to those which occur in the division of the infusorian "semen-capsule," now take place. There arise, namely, out of the simple circle of granules two circles, which move apart toward the ends of the spindle until they have finally arrived near the middle points of the future cleavage spheres; then the pointed ends of the spindle are usually no longer visible, and one sees only the two circles of granules and the fibres uniting them. Meanwhile the cleavage is nearly completed. When the formation of the nuclei begins, every distinct trace of the circles and fibres has *disappeared,* but what has become of them he does not know. Reasoning from the first-described spindle and its supposed origin from the equivalent of the infusiorian "nucleolus" (the germinative spot), Bütschli concludes that this spindle must owe its origin to the *nucleolus* of the primary cleavage sphere, although he was unable to recognize this nucleolus at any time previous to the appearance of the spindle.

Bütschli also observed that the new nuclei, even in the later generations, arise, as does the nucleus of the primary cleavage sphere, by the fusing of numerous nuclei which first grow from minute beginnings to a considerable size, and which in Cucullanus elegans arise at widely separated points. Likewise in Lymnæus auricularis there "arise eight or more small, vesicular, very clear nuclei, containing a number of dark corpuscles, which are not to be taken for nucleoli." These nuclei subsequently grow and successively unite to form a single nucleus,* —

* Oellacher (**'72^b**, pp. 406 – 416, Taf. XXXIII. Figs. 29 – 36) had already observed that the nucleus of segmentation spheres in the trout was composed of a cluster (as many as a dozen) of round or oval bodies varying somewhat in size, and containing each — at least during the first stages of cleavage — a single nucleolus. Oellacher

nucleus of the first segmentation sphere. The formation of the nuclei in this manner is proof to the author of the untenable position of those who, like Häckel, regard such a multinuclear structure as a complex of cells (p. 213).

Bütschli's omission of all reference to radial figures about the poles of the spindle is partly explainable from the transparency of the Cucullanus eggs, which prevents the rays becoming conspicuous, and partly from the great importance naturally attached to the newly discovered spindle.

Oellacher (**'74**) has described, in a paper which I have not seen, a radiate structure of the protoplasm as existing just before each act of segmentation in the case of the trout. I know only so much of the substance of this paper as is given by Flemming (**'75**, p. 207), according to whom, the radiate appearance is referred by Oellacher (just as by Fol and Flemming) to a structural condition of the plasm, not to a phenomenon of nuclear extinction.

Flemming (**'75**, pp. 117–128, 176 *et seq.* Taf. I.–III.), on the strength of renewed observations upon Anodonta and Unio together with a rotifer (Lacinularia), in which the entire absence of a nuclear structure during segmentation is maintained, accepts the views of Auerbach so far as regards the dissolution of the nucleus ("der morphologische Untergang des Kerns," p. 117), but presents numerous objections (pp. 188–198) to his theory that the radiate structure is due to a distribution of the nuclear sap from the tips of the nuclear cavity. He is "not yet altogether persuaded of the *karyolytic* nature of the radial figures" (p. 191).

considered each of these bodies as the equivalent of a cell nucleus, and explained the existence of a multiple of nuclei in each cell as a precocious activity of the nucleus, whereby it anticipated by several generations the division which ultimately overtook the protoplasm. Then with each segmentation half of the cluster fell to the share of each of the resulting protoplasmic elements. But to explain the continued recurrence of a large number of nuclei in each cluster, even after numerous segmentations, he was compelled to suppose that a process of multiplication was going on among the nuclei of these clusters, so that they, as it were, kept a definite number of generations ahead of the protoplasmic spheres to which they belonged, until at length, in the latest stages of segmentation, this difference becoming obliterated, one could find only cells with a single nucleus. However, Oellacher remains in doubt as to whether the multiplication of the nuclei takes place when there are still several in the cluster, or whether this only occurs when, by successive divisions of the protoplasm and corresponding separations of the components of a cluster, the nuclei have been reduced to a single one for each cell. No metamorphoses in these "nuclear clusters" were seen by him, and thus the possibility of a confluence of these "nuclei" before each segmentation was not, in that case, to be thought of.

Flemming finds that in Anodonta (Taf. III. Fig. 2), after the egg begins to elongate, previous to constriction, a disklike body, which stains deeply in carmine, occupies the middle of the clear figure connecting the two suns, and that a less intensely stained small spherical body occupies the centre of each sun. The latter he is inclined to think are the beginnings of the new nuclei, thus agreeing with Fol as to the *place* where the nuclei arise.

I believe the disklike body is almost unquestionably the *nuclear* plate, and not, as Bütschli (**'76**, p. 248) thinks, the *cell* plate.

The two radial figures, which are visible some time before the first segmentation, and which are of unequal size, (proportionate, namely, to the size of the two cleavage spheres that are about to be formed,) are no longer to be seen when the elongation preparatory to cleavage begins. Flemming is inclined to interpret (p. 128) the existence of single cells containing, as previously reported by himself, two nuclei, to be a pathological phenomenon, although stating as a possible explanation that it may not be of so much importance, after all, whether the new nucleus arises a little sooner (before division), or a little later (after cleavage).

In Lacinularia the only noticeable difference from Anodonta is to be found (p. 183) in the fact that the centres of the radial systems are not such distinctly limited clear spots as in the egg of the latter.

The primary (Keim) as well as later segmentation spheres divide while in the *cytode condition* (p. 184).

His (**'75**, pp. 35 – 39) contributes no new observations touching the question, Whence arise the parablastic cells? although he urges grounds against considering them derivatives from the cells of the germ layer. Indirectly, therefore, he implies that they arise *de novo* in the cortical layer of the yolk (Rindenschicht) and that their nuclei have not arisen by a process of division.

Soon after his last-mentioned paper, Bütschli published (**'75**[a]) further observations on the nucleus and its metamorphoses during cell division. The investigation of the contents of the testes in the case of Blatta resulted in showing that the nuclei of the multinuclear germ cells of spermatozoa did not undergo such a fusion as he had observed in the cases just reviewed, and as he expected to find here. The phenomena accompanying the division of the germ cells were, however, a striking repetition of the changes traced in his previous paper. In one point only does the author find reason to change his views. He now concludes that the spindle-shaped body results from the metamorphosis of the *whole nucleus*, not simply of the nucleolus. The nucleus suffers a con-

siderable reduction in size through loss of nuclear fluid (Kernsaft); it also loses its sharply defined membrane, although the existence of a delicate one around the spindle the author finds probable from analogy with the infusorian "semen-capsule." When the halves of the equatorial zone have reached the ends of the spindle, the surrounding protoplasm exhibits the radial structure already seen in other cases. Accompanying a constriction of the protoplasm the nuclear spindle assumes a bandlike appearance, with the dark granules of the zones occupying the ends of the band near the centre of the nascent cells. The constriction of the cell protoplasm is completed, and the two cells remain united only by the band. The formation of the new nuclei now begins by the appearance of a small, inconspicuous, clear space, filled with fluid, around the dark granular mass of the ends of the band. The dark granules pass into the interior of the new nuclei, and are the nucleoli. It is probable that the fibres of the band divide in the middle, and that the halves are absorbed into the corresponding nuclei. Very nearly identical results were also obtained in the study of the embryonic red blood-corpuscles of the common fowl.

The following conclusions are, among others, drawn by Bütschli: that the nucleus does not disappear in cell division, but only undergoes a very remarkable reconstruction (Neubildung); * that the karyolitic figure of Auerbach is most decidedly to be considered as the modified nucleus. A complete dissolution of the nucleus is not to be thought of.

It is evident from this paper that Bütschli now finds himself more at variance with the conclusions of Auerbach than previously. Although the author here refrains from expressing his opinion of the signification of the radial figures, he remarks that to a certain extent he agrees with Auerbach's views. Bütschli does not seem to have observed that these radial figures arise before the complete formation of the spindle, and does not mention them as existing till the time when the lateral zones reach the end of the spindle. His figures published by Strasburger show, however, that he had observed these stars before the division of the median zone.

* Lest "reconstruction" may appear an unwarrantable rendering of "Neubildung," I take the liberty to quote the context, which will show the author's real meaning. I must draw, says Bütschli, the following conclusions: "Dass der Kern bei der Theilung thatsächlich nicht schwindet, sondern nur eine höchst eigenthümliche Neubildung erfährt und dass die vielfach behauptete Neubildung der Kerne der Tochterzellen nur insofern dem thatsächlichen entspricht, als dieselbe eine Umwandlung des in so eigenthümlicher Weise modificirten Kernes in eine seiner ursprünglichen entsprechende Form ausdrücken soll." (p. 430.)

From Fol's ('**75**[a], pp. 108–112) memoir on the Pteropods one learns that the nucleus of the fecundated vitellus, or the germinative vesicle (as he still continues to call the primary cleavage nucleus), arises by the fusion of two or three corpuscles at the centre of a molecular aster whose rays disappear during the fusion. This nucleus, which attains sometimes a third the diameter of the whole vitellus, occupies the centre of the formative protoplasm; the latter is smaller than the nutritive portion of the vitellus, and the two meet in a plane. Although it shows a fine stippling, the nucleus is much more homogeneous than the protoplasm itself, and also less fluid. The nucleolus is always wanting. A nuclear membrane can be demonstrated easily after contact with sea-water or reagents, but this does not warrant the conclusion that it exists in the living condition.

After an interval of repose the nucleus disappears; but just before it ceases to be visible there appear on opposite sides, at the boundary between it and the protoplasm, two points, which differ but little from it in refractive power. Straight rays soon diverge from these points into its interior: they rapidly increase in number, and become elongated until those from the opposite sides meet in the middle of the nucleus, which at this moment disappears. No trace of rays is to be seen outside the nuclear vesicle in the living egg, but acetic acid causes also this portion of the aster to appear. If applied before the nucleus becomes invisible, the acid causes it, contrary to the case of Geryonia, to disappear.* The rays extend to near the periphery. The central part of each star is easily distinguishable without the use of acid, but is more distinct with it. The rays occupying the place formerly filled by the nucleus are often inflected, and pass from one star to the other. Soon after the nucleus disappears, the stars move apart. After a time a furrow is to be seen on the surface of the yolk running at right angles to a line joining them. Acetic acid develops, just before the appearance of the furrow, a very distinct line [plane?] of demarcation between the two stars. This line is formed of granules, which are a little larger than those of the rest of the protoplasm. As the furrow deepens and surrounds the yolk, it assumes a position oblique (in a constant direction) to the line joining the asters. It is during the approach and mutual flattening of the cells that the new nuclei appear at the centre of the protoplasmic part of each cell. Fol further states ('**75**[a], p. 180) that he has never yet seen segmentation preceded by a division, properly so called, of the nucleus, but would not

* P. S. — Fol ('**79**, pp. 219, 220) has since corrected and explained the cause of this error.

dare to assert that this mode does not exist among animals. Even in the case where the nucleus disappears, however, it in all probability forms none the less the central part of the molecular stars; and as it is in the centre of these stars that the new nuclei reappear, it may be presumed that the latter are, at least in part, composed of the very substance which constituted the nucleus before its division.

It is the origin of the new nuclei *at the centre* of the asters which is most strikingly in contrast with the results obtained in studying Limax. Fol also failed to recognize the fact that the deflected rays, which compose what is now known as the spindle, were in any essential respect peculiar. This oversight may have had its influence in preventing the author from giving an accurate account of the method in which the new nuclei arise.

The "line" of coarser granules may have been the first trace of the forming "Kernplatte," although no connection with the bent rays that pass from pole to pole of the spindle was indicated by the observer. Compare Fol's Fig. 5, Plate VIII., with Fig. 82 of Limax.

In another point there is considerable divergence between Fol's account and my own observations. In Limax the asters can be made visible by the use of acetic acid much earlier than is represented in the case of Pteropods. Might they not have been demonstrated by Fol for a somewhat earlier stage (e. g. for that shown in Fig. 2, Pl. VII.) by a more careful or prompt employment of acetic acid? If not, then we have to do in these cases with heterochronic variations. The comparison may be taken to afford ontogenetic evidence of a palingenetic concentration of the events of nuclear metamorphosis in the case of Limax. What the immediate motive to such an acceleration may have been, it is not easy to conjecture.

STRASBURGER (**'75**). The reader is referred to p. 372 for a synopsis of the results of Strasburger's studies, as the first edition of his work has not been accessible.

The first of a series of valuable articles by O. HERTWIG (**'75**) embraces the results of observations made chiefly on Toxopneustes lividus. The results obtained by the use of hardening reagents were controlled, as far as possible, by studies of the living egg. Around the nucleus of the first segmentation sphere (Furchungskern) the protoplasm has a radial arrangement which stretches to the periphery of the yolk, and in the immediate vicinity of the nucleus there gradually collects a homogeneous substance destitute of granules; furthermore, the nucleus itself undergoes a slight change of form, which is interpreted as the result of its amœboid motion.

Owing to both these changes the contour of the nucleus is less distinct than in the unfecundated egg. Its changes of form lead after a time to its permanent elongation. Its two poles are occasionally truncate, so that, in osmic acid preparations, it has the form of a cask. With this treatment the nucleus is homogeneous. Meanwhile the poles of the nucleus have become the centres of an accumulation of homogeneous substance, which forms at first a small area, and then enlarges in all directions. The formation and enlargement of the areas is accompanied (1.) by an arrangement of the yolk granules in rays directed toward the nuclear poles as centres, and (2.) by the growth of the suns thus formed by means of a peripheral elongation of their rays. The poles of the nucleus become in the living egg indistinct, and finally the nucleus suddenly disappears; but eggs treated with acids teach that the nucleus assumes the form of a bent spindle, each of whose tips appears as a conspicuous dark granule in the centre of its area. Later, the thicker middle portion of the spindle exhibits a number of dark, coagulated, intensely stained rods (Stäbchen), lying parallel to the long axis of the spindle, and appearing in optical section of the latter as a circular cluster of granules, which in Hertwig's figure (Fig. 27. *a* and *d*) appear evenly distributed over the whole area of the circle. From the intense staining it is concluded that these rods consist of condensed nuclear substance, on account of which they are collectively named the middle zone of thickenings (mittlere Verdichtungszone). This structure is referable to a process of differentiation in the nucleus similar to that which takes place in the formation of nucleoli (p. 414).

After the apparent dissolution of the nucleus, the two suns are connected by a narrow non-granular band, which occupies its place (dumb-bell figure); the peripheral ends of the rays extend either to the surface of the yolk, or to a plane (Theilungsebene) perpendicular to the middle of the band, and the deep ends approach the centres of the suns with such want of uniformity as to give the homogeneous area an irregular outline.

The nature of all such "Radienfiguren" is explained (pp. 415, 416) as resulting from a force which is exercised by the nucleus and expresses itself in an attraction of the homogeneous protoplasm, so that the radial arrangement of the yolk granules is only the optical expression of the disposition of the protoplasm in which they are imbedded. The granules are replaced by the protoplasm in the vicinity of the nucleus, since the latter exercises no attractive influence upon them. The attractive force, at first operating uniformly in all directions about the "Furchungskern," is distributed at the time of the lengthening of the nucleus to its

poles, and increases with the increase in the distance between the poles, attaining its maximum in the dumb-bell stage, after which (at division of the nuclear band) it wanes and altogether disappears. The elongation of the nucleus and the radial figures are together comparable to the magnetic rod and its influence on iron filings, without, however, necessitating the implication of an identity of forces.

About the time the nucleus begins to elongate, the outline of the egg undergoes for a short time a series of changes in form (pp. 403, 404, 417), consisting of low elevations. In the dumb-bell stage the egg elongates in the direction of the handle of the dumb-bell, and a circular furrow appears in the plane of division and finally effects the separation of the halves. This constriction is often accompanied by irregular changes of the surface in the form of lobed pseudopodial processes, which here and there arise and disappear. This phenomenon presumably has a causal relation with attractions in the nucleus.

The further changes, as seen in the interior of the living egg during and after the constriction of the yolk, begin with a separation of the heads of the dumb-bell figure, and a modification of their form. Each is at first flattened in a plane parallel with the division plane, and then becomes a meniscus, the concave surfaces of the two meniscuses facing each other and continuing in connection by a thin pedicel. About the time the constriction divides the yolk, there suddenly appears a small clear spot in each half of this pedicel, at some distance from the division plane, and this spot, which is at first of irregular form, becomes rounded and increases in size; it is the nucleus of the daughter cell. Next, the radial arrangement of the yolk disappears, then the pedicel vanishes, the nucleus migrates partly into the meniscus, and finally the latter is reduced to two small areas at the sides of the nucleus. The newly formed nuclei in this, as well as in all subsequent stages, are without membrane, and consist of a homogeneous substance. Inasmuch as the nuclei are of nearly uniform size for the first few generations, it follows that a considerable increase in the nuclear substance has taken place after each segmentation.

Osmic acid preparations stained in carmine furnish additional information on these internal changes. Preparations of eggs somewhat older than those exhibiting the "middle zone" show a ribbon-shaped body in its place. Its ends reach into the middle of the suns, and, owing to the intensified action of the reagents, appear as dark sharply defined streaks. Where the band enters the head of the dumb-bell it presents rodlike thickenings (seitliche Verdichtungszone des Kernbandes).

Between these zones lies the "middle piece"; beyond them the "end pieces." Each of these is homogeneous, slightly reddened, and only rarely striate in osmic preparations; but in chromic acid preparations fine streaks are seen to connect the rods of one lateral zone with those of the others. During the constriction of the yolk, the nuclear band lengthens, the two lateral zones continue to move apart, and lose their striate differentiation. In place of the rods are larger or smaller granules, and drops which have arisen by a confluence of granules, or it may be a single dark red mass with a knobbed surface. The end of the band is broadened, and its corners are drawn out into two prolongations (Spitze), which appear as dark granules. After the completion of the constriction, the lateral zones gradually become thicker and finally assume the spherical form, and the middle and end pieces become shorter and disappear by uniting with the rest of the nuclear mass. Thus the nuclei of the daughter cells arise in the parts of the nuclear band called lateral zones.

These phenomena may in their interpretation be divided into two groups, says Hertwig; the one relating to the changes of the nucleus, the other to those of the yolk. They accompany each other in such a manner that each form of the nucleus corresponds to a definite method of arrangement of the protoplasm, so that an intimate connection between the two must be inferred.

In considering whether the impulse to division proceeds from nucleus or protoplasm, Hertwig says that it is from the former, and "therefore considers the nucleus as an automatic centre in the cell equipped with active forces." The lengthening of the nucleus is to be considered, like its earlier amœboid changes, as the result of active phenomena of motion on the part of the nucleus, yet with this distinction, that the displacement of particles is now only in two directions, instead of in all directions. The two poles of the nucleus exert a repulsive influence upon each other, and determine the distribution of the remaining nuclear mass. The two lateral zones arise out of the middle zone,* and migrate

* A statement made by Priestley ('76, p. 152) in his review does not seem to reflect in a very accurate manner the ideas of Hertwig as to the connection between the middle zone and the nuclei of the newly formed cells. The sentence in question is as follows: —

"Although Hertwig in his hardened and stained specimens does not certainly speak of the derivation of the young nuclei from the first *median thickened zones* [*zone*], there can hardly be a doubt that the *lateral thickened zone*[*s*] (which afterwards became the nuclei) correspond entirely to the segments of the nuclear disk described by Bütschli, Strasburger, and Beneden, and resulted from division of the former zone." If,

toward the ends of the nucleus without fully reaching them. As the rods (Stäbchen) of the middle zone arise by a differential process which separates "nuclear substance" from "nuclear sap," so the new nuclei arise by the reverse process, — the rods imbibe nuclear sap, swell, and form granules which melt together to constitute the nuclear mass. A condensation of nuclear substance is likewise the cause of the dark granule at the end of the spindle and the dark streak at the end of the nuclear band. The "Radienfiguren" consist in a radial grouping of the protoplasm around the nucleus or definite points of the same.

It is concluded, further, that "the division of the nucleus is a process entirely independent of the division of the protoplasm," since in certain eggs, which probably had gradually succumbed to the effect of external noxious influences, the division of the nucleus was not followed by a corresponding process on the part of the protoplasm.

The two main results which Hertwig deduces from the foregoing are: — (1.) In egg segmentation a dissolution of the nucleus does not take place; the nuclei of the segmentation spheres are rather parts (Theilstücke) of the maternal nucleus. The supposed disappearance of the nucleus before division is explainable from its peculiar changes of form, by reason of which it becomes less easily recognizable in the living object. (2.) In cell life a high physiological significance belongs to the nucleus, for it must be considered as an automatic force-centre. In cell multiplication this especially comes into activity, inasmuch as it impels and regulates the same.

For the most part I can only confirm the views entertained by Hertwig; in one or two points, however, I find a difficulty in adopting his opinions. The amœboid changes of form which the nucleus presents are probably referable to an inherent activity of the nucleus, which warrants the conclusion that it is an automatic centre in the cell; it seems less certain that the elongation of the nucleus and the appearance of two centres of attraction are referable to the same force resident in the nucleus. In the first place, only two substances are recognized as entering into its composition, — a nuclear sap and a nuclear "substance." The latter is the active component. If, as Hertwig seems to infer, that part

as seems to be the case, Priestley entertained the opinion that Hertwig had failed to draw and to formally express a conclusion establishing a genetic connection between the new nuclei and the median zone, then it is probable that the following passage must have been overlooked by him: "Aus der Aufeinanderfolge der verschiedenen Bilder glaube ich den Schluss ziehen zu dürfen, dass die beiden seitlichen Verdichtungszonen aus der mittleren entstanden sind." (Hertwig, p. 414.)

of the nuclear "substance" which is accumulated at the *poles* of the elongated nucleus exerts an attractive influence on the surrounding protoplasm, it seems only natural to inquire why the greater mass, accumulated at the equator, does not exercise a like influence, and why it is that the latter seems to respond quite as passively as the protoplasm to the attractive influence of the centres of the asters. I am inclined to think that the astral phenomena are not to be explained so simply as by the assumption that they are due to the attractive influence of a segregated portion of the nuclear "substance" as such; but that it is more likely they arise in response to a force set free by rapid chemical changes at definite points in the protoplasm. From the usual proximity of those points to the nuclear structure, it seems highly probable that the chemical changes are sustained by the direct mingling of the substance of the nucleus with the protoplasm; there are some cases, however, (e. g. Limax,) where the evidence of a direct mingling is wanting, — where the early participation of the substance of the nucleus may possibly be called in question.

Hertwig maintains the morphological integrity of the nucleus throughout the metamorphosis. If such is the case, are we not justified in expecting that its attractive influence will continue to be exerted from the beginning to the end of the process? In that event, the two asters which arise with the lengthening and polar differentiation of the nucleus ought to be genetically connected with the *single* aster which first radiates from the "Furchungskern." Selenka, it is true, has recently maintained this very position, but I believe that Hertwig (p. 416) is right when he practically denies the existence of a genetic connection in saying: "Dann löst sich die alte Radienfigur allmälig auf und es entstehen zwei *neue* an den beiden Polen des Kerns. Dieselben sind anfangs klein, u. s. w." Hertwig believes the amphiaster becomes explainable as the result of the attractive influence of the nucleus on the protoplasm, as soon as we assume that with the lengthening of the nucleus there is a distribution of the forces of attraction to its poles.* I find nothing to support this conclusion, since I see no evidence of a genetic connection between the single and the double asters. Both may be the result of like chemical changes, but certainly the latter are not referable to the direct attraction of the nucleus, or a segregated portion of it.

* "Auch diese Erscheinungen erklären sich aus einer Anziehung, welche der Kern auf das Protoplasma ausübt, wenn wir annehmen, dass die zu Anfang in der Kernkugel nach allen Richtungen gleichmässig wirkenden Anziehungskräfte bei der Streckung des Kerns auf die zwei Pole desselben sich vertheilen."

I am not prepared, then, to grant that the "Anstoss" to the processes of cell division proceeds from the nucleus; nor does it seem to me imperatively necessary to accept the other horn of the dilemma offered by Hertwig. From purely *a priori* considerations, one might be inclined to think the initiative lay with the protoplasm, for in that event the division of a *cytode* would not demand a special explanation different from that of *cell* division; but with the view I have suggested it is perhaps as inappropriate to inquire which takes the initiative as it would be to ask whether the carbon or the oxygen begins the process which results in the production of heat.

There is ground for believing that in Limax the whole of the "middle piece" does not enter into the composition of the new nuclei, but that portions of the interzonal fibres remain permanently near the surface of the vitellus.

The flattened or band-like condition of the spindle I have not seen; it is probably of rather limited occurrence. I have not been able to discover Hertwig's so-called end-pieces, or, to be more exact, I have not seen the nuclear fibres reach the centre of the sun. This, after all, varies with the particular stage of advancement, for practically the rays and spindle fibres in the beginning reach almost to the centre of the aster. It is in the later stages (when, for example, a confluence in the elements of the lateral zones has begun) that I fail to find evidence of the continuation of the fibres beyond the region of the lateral thickenings.

In his preliminary note on the development of Heteropods, Fol (**'75**[b], p. 472) says: "Here also the nuclei disappear before each segmentation, and are replaced by molecular stars."

In his history of the development of Bombinator igneus, Goette (**'75**) describes the events which succeed the fecundation of the egg substantially as follows.

After the disintegration of the germinative vesicle there arises, probably near the centre of the yolk, a "vitelline nucleus" (Dotterkern) which is not histologically distinguishable from the surrounding yolk. Between this nucleus and the finely granular substance left behind by the germinative vesicle there is only a chance [i. e. no genetic] relationship (p. 51). Owing to the absence of coarser yolk corpuscles (Dotterplättchen) from this nucleus, and to the dark color of the finely granular substance which takes their place, the outline of the nucleus is visible. It migrates toward the surface of the yolk, whereupon there arises within it a delicate round corpuscle, — the first "life-germ" (Lebenskeim). The vitel-

line nucleus is, in Goette's opinion, certainly the initiatory point of the whole development. Subsequently its outline melts away, and with it the significance of the nucleus itself; the "life-germ," however, remains, and surrounding it — though not sharply marked off from it — an area of fine-granular yolk substance, which is not constant in size, nor in the sharpness of its limitation from the remaining yolk (pp. 54, 55).

Soon after its formation the life-germ is elongated in a direction transverse to the axis of the egg. Its ends enlarge at the expense of the middle portion, which continues to grow more slender as the ends diverge from each other. It at length breaks, and the halves become rounded, having meantime begun to increase, so that eventually each attains the size of the first "life-germ." As observed in subsequent life-germs, the elongation results from a change in the form of the germ, whose remaining axes shorten, and is not, therefore, due simply to a growth at two opposite points (pp. 55, 60). From what is said of the division of the *first* life-germ (p. 55), one would infer that the changes of the surrounding "area" *followed* those of the germ; but it is distinctly stated in another place (p. 61) that this finely granular substance of the area *initiates* the motion in two opposite directions, itself dividing into two masses before the germ does.

After the second division of the yolk, there is an essential change in the contents of each life-germ; namely, a variable number of round, clear corpuscles appear in the apparently homogeneous germ-substance, which are the "nuclear germs" (Kernkeime). These are so soft that they become elongate at the subsequent division of the life-germ. While the Kernkeime augment and thereby consume the substance of the latter, it, together with its surrounding "area," melts into a single delicately granular mass, in the middle of which the compacted Kernkeime fill up the space which the original life-germ had occupied.

As the *nuclear* germs supersede the *life*-germ spatially, so they do functionally in the subsequent divisions of the yolk. During the mulberry stage the division occurs so rapidly that the nuclear germs have not time to attain the centre of the finely granular mass before the latter has begun to elongate preparatory to the next division, which is usually effected along a plane perpendicular to that of the last preceding. At the close of the division the fine-granular mass appears radially streaked about the nuclear germ [aster], and likewise delicate dark lines converge from the plane of division toward each of the germ masses [spindle]. New nuclear germs constantly arise, not so much by division as probably out of the amorphous fine-granular mass, and associate themselves with those already formed.

Such, according to this author, is the nature of the process for only a limited number of segmentations, however. Toward the end of yolk cleavage — when the segmentation begins to be no longer easily discerned by the unaided eye — the cluster of nuclear germs (Kernkeimhaufen) melts together into a solid corpuscle, which retains for some time an irregular outline and a netlike pattern, — the last trace of its composition out of separate nuclear germs, but which eventually becomes a sharply defined, round, finely granular "cell nucleus." The division of the cell nucleus results from a one-sided outgrowth, not from an elongation, as in earlier segmentations.

It is impossible to review here in detail the extended reasoning put forward by Goette to show that neither the unfertilized nor the fecundated egg are living substance, and how that, during the yolk division, both the whole yolk sphere and the individual yolk masses [segmentation spheres] are lifeless stages of transition from unorganized matter to an actual [living] organism (p. 77).

Having discovered that the physico-morphological conception of the cell is incompetent to stand for a complete definition, he seems to fall into an equally exclusive method of reflection, and denies that the egg is a cell because it fails to exhibit to him one of the functional peculiarities of living things. It does not live because he discovers no process of nutrition (Ernährung), and that there is no such thing as a nutritive function is confirmed, sufficiently for him, by the single fact that the egg does not grow, — does not increase in size, except in a manner quite foreign to the method of growth in living cells. Thus denying the adequacy of the morphological conception, he will not even allow any other functional manifestation than that of growth to stand in evidence for the living condition of the egg, or its real cell nature. The motive to such a theory of the transition of lifeless to living matter has, to say the least, not been strengthened by the accumulating evidence of a genetic connection between the germinative vesicle and the subsequent generations of nuclei.

According to Goette, the formation of the "life-germ" and its area is only the result of a radial diffusion (p. 88), the optical expression of which has already been noted, and the life-germ, in its turn, causes in a certain way the continuation of this diffusion. The only essential difference between the developing and the not-developing egg lies in the regulation in one case, and the want of regulation in the other case, of the osmotic process which takes place in both instances between the yolk and the water surrounding it.

There is one point in Goette's account which seems to me corroborative of the views I hold about the nature of the asters. The fine-granular area surrounding the nuclear germ elongates before the latter undergoes any change of form. Whitman (**'78**[a]) has observed a similar instance in the case of Clepsine, but has interpreted the elongating area as a *nucleus.* I believe that Goette's *nuclear germ* and Whitman's *nucleoli* are the same, and are really *nuclei*, and consequently that the "granular area" of the one and the "nucleus" of the other are both cell protoplasm, so that in these two cases the first optical evidence of a coming division is manifested, not in the nucleus, but in the protoplasm immediately surrounding it.

Very little attention is bestowed by Kowalevsky (**'75**, pp. 609, 610) on the changes in the nucleus and cell protoplasm during segmentation, as observed in Pyrosoma. The first division of the formative yolk is effected by a furrow, which, beginning on one side, sinks deeper and deeper, and near which is observed in each segmentation ball a nucleus of stellate form (Fig. 13). An examination of the figure is sufficient to convince one that the author has overlooked the real nucleus, and has taken therefor the stellate figures in the protoplasm of the cell. The reader will recall the fact that Kowalevsky had previously (**'71**) maintained that the stellate structure was limited to the *nucleus*, and will not be surprised to find that this opinion has caused him to portray these figures with an abruptness of outline (Fig. 14) which is not often seen. The rays in the figure alluded to are even made to terminate distally in slight enlargements.* Nothing in the figure would indicate that this observer saw anything of the spindle-shaped condition of the nucleus, or of the nuclear plates, which is the more surprising, if staining was resorted to for these earlier stages, as it certainly was for later ones.† The author thinks it probable that a division of the nucleus precedes that of the yolk, although he has not directly observed it.

* Although Eimer (**'77**, Figs. 13, 18, etc.) has recently shown that similar nuclei are found in tissue cells, and especially in Cœlenterates, I am still inclined to think that Kowalevsky's "nuclei" are stellar arrangements of the cell protoplasm, such as exist in the case of many other animals.

† If I am wrong in considering these radiate lines as belonging to the protoplasm rather than to the nucleus, then they probably can only be considered as the spindle fibres of a nuclear spindle seen endwise, much as depicted by Strasburger (**'76**, Taf. VII. Fig. 18 *a*). There is, however, a serious objection to this explanation, for two of the cells in Fig. 14 (Kowalevsky) are in an advanced stage of segmentation, and are so located, with respect to the observer, that the spindle could have been seen only *en face*, — not end-wise !

In a stage somewhat later than the mulberry stadium, a section of the embryonic cell-mass — which is grouped about one pole of the egg — shows (Fig. 17) in each cell a nucleus (about which the protoplasm in the figure is shown to have a faintly radiate arrangement), with one or two nucleoli, and numerous cells in process of division (p. 610). Between the cells of uniform appearance there are found a few which differ from them in being smaller, more intensely stained, and in possessing very little protoplasm about the nucleus. Whether these latter are identical with the "cells in process of division," the author does not state.

Notwithstanding the admitted insufficiency of his observations on the development of the eggs of the rabbit, ED. VAN BENEDEN (**'75**, pp. 704, 705) believes he may affirm that the supposed [nuclear] vacuoles, which, according to Auerbach, appear in the karyolytic figure during the first segmentation, are not newly formed elements, but fragments of the first embryonic nucleus, which has changed from a spherical to a fusiform condition. They (vacuoles) are bodies formed from nuclear substance; they become rose-colored in picrocarmine. Each sphere, at the end of the first segmentation, presents a regularly spherical form, and discloses a clear spot composed of two distinct parts. The smaller one, derived from the first embryonic nucleus, is called *pronucleus dérivé;* the larger, with a bunched surface, and incompletely enveloping the smaller one, is named *pronucleus engendré.* The latter is only the remnant of the homogeneous, transparent substance accumulated in the first sphere at the two poles of the first nucleus after the latter has taken the form of a spindle. It is a differentiated portion of the protoplasm of the cell in process of formation, and presents no genetic bond of connection (lien) with the nucleus of the first sphere. The *pronucleus dérivé* grows at its expense, finally absorbing it completely. The "derived pronucleus" thus becomes *the* nucleus of its vitelline sphere, and contains numerous refringent nucleoli.

The spheres, some time after division, lose their spherical form, and become mutually flattened, in which state the "pronuclei" have given place to a single nucleus.

Chapter VI. of this preliminary communication is devoted to cell multiplication. The *résumé* of results from the study of the ectoderm cells, in the case of the rabbit (pp. 732–736), do not differ very materially from the results of Bütschli and Strasburger. The first phenomena which announce the approaching division of a nucleus have their seat partly in the nucleus itself, and partly in the body of the cell. The

contour of the former becomes indistinct, and its form irregular, owing, possibly, to its amœboid movements. The nucleoli disappear. The substance of the nucleus is soon divided into two parts : the one, which is clear and transparent, and which is not colored in either carmine or hæmatoxylin, is the *suc nucléaire;* the other, which is likewise homogeneous, but which becomes deeply stained, and forms an irregular lump (grumeau) in the middle of the nucleus, is the *essence nucléaire.* The nuclear sap accumulates at the two poles of the elongated nucleus; the "essence," at the middle of the nucleus, to form the equatorial plate. The faces of the latter are bunched, and consequently irregular. It seems formed of very refringent globules of an ovoid, or of a rod-like form. Whatever the method of treatment, the nucleus was never found at this time to be striated, either longitudinally or transversely. The body of the cell undergoes concomitant changes of form; it also becomes more granular, and slightly stained by coloring fluids, these latter peculiarities serving to distinguish at once the cells in process of division.

The nucleus becomes spindle-shaped, then flattened (rubané). At its poles there accumulates, in the body of the cell, a little clear, finely granular substance, which the author hesitated to identify with his *pronucleus engendré,* though he would at present probably not entertain any doubt as to the correctness of this identification. This polar mass becomes the centre of a stellate figure developed in the protoplasm of the cell, and indicates very manifestly the attraction exercised by the poles of the old nucleus upon the protoplasmic substance of the cell. These stellate figures, already seen by numerous observers in segmentation spheres, have not been previously pointed out, so far as the author knows, in *ordinary* cells.

The equatorial granular plate divides into two parallel *disques nucléaires,* which separate as though mutually repulsive. These two plates are connected by filaments (Kernfäden of Strasburger), which appear to be thrown out by some of the granules which constitute the disks. After the disks have separated from each other, these filaments are drawn in, and blend with their substance. Meanwhile, the nucleus takes the form of a band with parallel edges, and the nuclear sap (very faintly rose-colored in picrocarmine), which had at first been repelled to the poles of the nucleus, is accumulated between the two disks. The latter finally reach the extremities of the nuclear band, and come into immediate contact with the small, clear mass at the centre of the stellate figures. The clear band, which is the remnant of the old nucleus,

is now composed of the two disks and a *pièce intermédiaire*, the latter being only slightly, or not at all, stained. During the constriction of the body of the cell, which now takes place, and which does not encroach upon the band, there occurs in the *pièce intermédiaire* a differentiation of substance at the niveau of the constriction. Treatment with nitrate of silver causes the appearance of black points, which become more and more numerous. These at length become aligned, and form the partition separating the two produced cells. The parts of the intermediate piece adjacent to the partition blend more and more with the cortical zones of the produced cells; the part adjacent to the polar disk becomes, on the contrary, granular, and gradually blends with the medullary mass of the cell. The polar disk becomes, as maintained by Strasburger, the nucleus of the produced cell, — not the nucleolus, as claimed by Bütschli. It appears to enlarge at the expense of the small, clear mass to which it is joined, after the corpuscles which form it are united into a homogeneous mass; the latter takes an oval form, and becomes more and more regular. The substance of the young nuclei are stained less by carmine and hæmatoxylin as the cell enlarges. The body of the cell soon ceases altogether to be stained.

A *résumé* of this paper, together with those of Auerbach (**'74**), O. Hertwig (**'75**), and Strasburger (**'75**), has been published in the Quarterly Journal of Microscopical Science, by Priestley (**'76**).

Auerbach (**'76**) objects to considering the "Kernspindel" as equivalent to the nucleus. It corresponds to the middle part of the karyolytic figure,* for the following reasons: —

(1.) Its volume is usually greater than that of the nucleus.

(2.) It does not have a sharp, but rather a very confused, limitation.

(3.) It is to be found only at the time of, or after, the disappearance of the old nucleus. It demands the use of chemical reagents to make a differentiation within its substance apparent. This structural appearance is the optical expression of regulated morphological conditions under which the commingling, and subsequently the separation, of the two substances proceed, — the expression of inequalities in their distribution, — and indicates, on the other hand, those molecular displacements which are incident upon the progressive elongation of the whole (figure). Toward the end of the process there is formed in the equatorial plane, owing to an elimination of the nuclear sap in the direction of the poles, a more

* "Der bewusste längsstreifige Körper ist nicht der Mutterkern, sondern der Mittheil der von mir sogenannten karyolytischen Figur, also ein Product der Vermischung der eigentlichen Kernsubstanz mit dem umgebenden Protoplasma."

compact transverse layer. This persists, and as a wall of separation prevents the fusion of the two new nuclei which arise near each other in the handle of the dumb-bell figure.

(4). It is not fully, nor even principally, composed of "Kernsubstanz," for its principal mass does not enter into the formation of the new nuclei.

Furthermore, the new nuclei do not arise by the division of a mother nucleus. The substance of the striate body (spindle) does not enter into the formation of the new nuclei, but the latter are differentiated *only at the poles of the spindle body*, as two relatively small, spherical, clear, and homogeneous bodies, which, at times, are seen to be formed by the confluence of smaller drops, and therefore evince their origin as collections of previously distributed substance. The greater portion of the spindle structure is not transferred to the new nuclei, but merges with the protoplasm, and even lies in the periphery of the daughter cells, where it helps to form (plants) the cellulose membrane. The striate body is a structure combined out of nuclear substance and cell protoplasm, which latter has made its way in from the sides.

The evidences of the formation of the spindle within the still persisting nuclear membrane (see especially O. Hertwig's studies) are too numerous and unequivocal to allow any doubt in the cases presented. I cannot but believe, however, that the exact size of the spindle, as compared with that of the nucleus, has little to do with the question of a dissolution of the whole nucleus. A total disappearance of the old nucleus is, of course, the cardinal point. I have never found a stage in which nuclear substance was not demonstrable by staining, and agree with those writers who derive the new nuclei primarily from the halves of the nuclear plate, — I even doubt if the poles of the spindle (or the corpuscles of the central "areas") take any direct share in the composition of the new nuclei.* Of this latter point, however, I am not fully persuaded. It is possible that the increase in the size of the young nucleus may ultimately bring its periphery in contact with these corpuscles, while the latter are still intact, and that they may then directly contribute to the formation of a nuclear membrane. I am, however, more inclined to think the corpuscles cease to exist as discrete structures before any such event could transpire, and that they contribute less directly, if at all, to the substance of the nuclear mass.

Another paper by Auerbach, directed principally against views entertained by Strasburger, is considered in another connection (p. 370).

* See also the objections raised by Flemming (**'78**[b], p. 415).

In his work on the development of Elasmobranch fishes BALFOUR ('76, pp. 393–402, and '78, pp. 15–24, Pl. II.) has figured and described some interesting observations on the changes of the nucleus during segmentation. The earlier stages of cleavage did not afford information in this direction, but when the segments had become numerous, and in diameter between 0.25^{mm} and 0.08^{mm}, it was observed, in sections of specimens hardened in chromic acid, that the place of the nucleus was occupied by a sharply defined figure having the shape of two cones placed base to base, which stained as deeply as a nucleus. "From the apex of each cone there diverge toward the base a series of excessively fine striæ. At the junction between the two cones is an irregular linear series of small deeply stained granules, which form an apparent break between the two. The line of this break is continued very indistinctly beyond the edge of the figure on each side. From the apex of each cone there diverge outwards into the protoplasm of the cell a series of indistinct markings. They are rendered obscure by the presence of yolk spherules, which completely surround the body just described, but which are not arranged with any reference to these markings."

The course of the markings (*loc. cit.*, Pl. II. Fig. 7 *a*), which evidently correspond to asters, is quite unlike anything observed by others; if not fundamentally, at least in the extent of the deflection which the lines suffer, causing, as it does, the most of them soon to take a course almost parallel with the sides of their respective cones. I recall nothing similar to this appearance, unless possibly the fibres which Strasburger ('76, p. 45) describes as resulting from a resolution of the "mantle" of the cask-like spindle into filaments in Spirogyra may be comparable to it. In the present case, however, the "markings" do not form fibres continuous from pole to pole. Balfour justly calls attention to the fact that the striæ of the cones are not to be confounded with the markings, for the cones are quite as distinctly differentiated from the cell as nuclei are, whereas the "markings" are merely structures in the general protoplasm of the cell.

The end view of the spindle given by Balfour (Fig. 7 *b*) affords no idea of the real distribution of the "linear series of granules," which represents the nuclear plate. The colored circular body in each cone, which he once observed, — after a line indicating cell division had made its appearance in the plane of the base of the cones, — may possibly represent the lateral zones of thickenings; the drawing (Fig. 7 *c*), however, leaves room for doubt.

The important observation is made by Balfour that these conelike

bodies are not only found in the cells of the germinal disk, but also in the yolk *completely outside* the disk. From this the author concludes that these bodies can occur where their connection with cell division is altogether out of the question, — where, in their changes, they are without any influence on the surrounding protoplasm.

In later stages of segmentation the conelike bodies are less frequently met with, notwithstanding the fact that the nuclei are more numerous, and are increasing in number more rapidly than during earlier stages. Other bodies are seen, however, which are intermediate between them and ordinary nuclei. Of the three figures (8 *a–c*) given of these intermediate structures, one (8 *a*) is evidently a spindle in which the nuclear plate is about dividing, and another (8 *c*) is a more advanced stage, while the third is not so easily referable to known stages in the metamorphosis. Balfour affirms that the granules contained in these bodies exactly resemble the granules of typical nuclei. All these bodies occupy the place of, and stain like, ordinary nuclei, and are as sharply defined. The true nuclei of the germinal disk are for the most part regularly rounded; those of the yolk are often irregular in shape, and provided with knoblike projections, indicative of a process of division in the primitive nucleus.* In no case is a distinct membrane to be seen around any of the nuclei.

Balfour's conclusions, drawn from a comparison of his results with those of other observers, may be reproduced in his own words: —

"In the act of cell division the nuclei of the resulting cells are formed from the nucleus of the primitive cell. This may occur, —

"(1.) By the complete solution of the old nucleus within the protoplasm of the mother cell, and the subsequent reaggregation of its matter to form the nuclei of the freshly formed daughter cells;

"(2.) By the simple division of the nucleus;

"(3.) Or by a process intermediate between these two where part of the old nucleus passes into the general protoplasm and part remains always distinguishable and divides; the fresh nucleus being in this case formed from the divided parts as well as from the dissolved parts of the old nucleus."

A series of all possible gradations between the first and second, may be embraced under the third.

Balfour's conception of the cause of the stellate figures is more mechanical than that of Auerbach, whom he in the main follows. The streaming out of the protoplasm of the nucleus into that of the cell will

* It is more likely that these projections are stages in the confluence of several vesicles to constitute a nucleus.

be accompanied by the formation of a wake on the peripheral side of such large granules as cannot be displaced. Any granules carried by the current into this wake will remain there, and thus contribute to the formation of a radial row of granules, and a series of such rows would produce an appearance of striation.

The conclusions drawn for Elasmobranchs are, that in the earlier stages of segmentation, and during the formation of fresh segments, a partial solution of the old nucleus takes place, but all its constituents serve for the reconstruction of the fresh nuclei; that in later periods of development a still smaller part of the nucleus becomes dissolved, and the rest divides, but that the two fresh nuclei are still derived from the two sources; and, finally, that after the close of segmentation the fresh nuclei are formed by a simple division of the older ones.

Ludwig ('76, p. 484) believes that in the development of spiders' eggs the increase in the cells may be accompanied by nuclear changes such as have been observed by Fol, Bütschli, and others. Evidence of this is to be found especially in the radial direction of the deutoplasmic elements in the separate cell territories and the appearance of the nucleus in the centre of this radial structure, as well as in the fibre-like (strangartig) connection of two recently separated centres.

The origin of the nuclei (p. 476) was not satisfactorily made out. In one case the nucleus seemed to arise by a fusion of a number of small round structures, in which case the central mass assumed a foamy appearance which disappeared as soon as the nucleus became visible. One might conclude that the nucleus arose by a fusion of the vacuoles which caused the foamy appearance, were it not for the fact that the vacuoles were sharply — the nucleus only indistinctly — outlined.

The fusion of the deutoplasmic balls into columnar structures, and the radial arrangement of the latter (p. 474), the author refers not to any subjective activity of the deutoplasm, but considers as a passive phenomenon brought about by the active vital processes in the protoplasm.

In his book on *Zellbildung und Zelltheilung*, Strasburger ('76) availed himself of the then recently made observations on cell division by Auerbach, Bütschli, and others, to show the prevalence of the phenomena observed by him to take place in plants. To these evidences he adds (pp. 208–231) observations of his own. Those relating to the changes occurring in the cell division of cartilage are considered in another connection. His other observations were made on the eggs of Phallusia mammillata and Unio pictorum.

In artificially fecundated eggs of Phallusia the nucleus of the first

segmentation sphere immediately upon its formation is surrounded by a homogeneous "Plasmazone," and by rays traversing the contiguous granular protoplasm. The rays increase in length as the nucleus, with gradually retarded velocity, moves from the periphery to, or near to, the centre of the egg. The nucleus, at first homogeneous, increases in size after its migration, and becomes less refractive; the rays and "Plasmazone" disappear. The nucleus becomes indistinct, and it can be shown, by the use of reagents (p. 213), that it becomes spindle-shaped, streaked, and acquires an equatorial "Zellplatte" [*Kern*platte?]. However, in *living* eggs (p. 217) one can see that the arrangement of the plasma becomes radial to the two poles of the nucleus about to divide, and more distinct as soon as the segments of the nuclear plate separate, — Auerbach's karyolitic figure. If, at the beginning of the division, the nucleus remains eccentric, the whole figure moves toward the centre of the egg. The "Kernfäden" [interzonal filaments] are not numerous, and become indistinguishable before completed division.

It may be observed in Limax that the filaments remain visible longer. I think their early disappearance in Phallusia may have been due to the nature of the reagent (osmic acid) used by Strasburger, rather than to any difference in the eggs.

The rays increase in length as the formation of the new nuclei advances, till they reach the periphery and equatorial plane; the egg lengthens in the direction of the line uniting the new nuclei; the equatorial plane becomes clearer. An annular constriction, beginning on all sides at the same time, appears, and the division is accomplished so quickly as to seem simultaneous throughout its whole extent. The rounded segmentation products become mutually flattened.

The nuclear metamorphosis, as demonstrated by reagents, is the same as in Unio (see below). The author believes he has now and then seen slight thickenings (Zellplatte) in the equator of the interzonal filaments similar to those observed in plant cells, and thinks they may arise from the "Hautschichtmasse" collected in the plane of division; he also suggests a similar interpretation for Fol's figure of the Pteropod egg, but, as has already been indicated (p. 292), it is probable that the figure in question represents a stage antecedent to the formation of the *nuclear* plate, and hence can hardly bear the interpretation proposed by Strasburger. As the second division is being initiated, it sometimes happens, under abnormal circumstances, that the two segmentation cells formed by the first division again fuse. In eggs thus artificially made unicellular, there may be distinguished four suns united in pairs by connecting bands.

Owing to the opacity of the cells in Unio (pp. 213 – 216) they were studied with the aid of sections. Only embryos consisting of about twenty cells were used. The nuclear plate is found by study of cross sections to be formed, not of an annular series of granules (Körnerkranz), but by a continuous disk (durchgehende Körnerscheibe). The author does not mention any inequality in the distribution of the granules, such as is exhibited in Limax, nor does his figure (*loc. cit.*, Taf. VII. Fig. 18 *b*) make the granules of the "Kernplatte" more conspicuous than those of the surrounding protoplasm. The poles of the spindle are much more marked, on account of their great refractive power, than in plant cells. The rays of the protoplasm converging toward them are very distinct in animal cells, hardly traceable in plant cells. The two segments resulting from a division of the nuclear plate are found in different cells at varying distances from each other. Interzonal filaments are neither increased in number, nor suffer a lateral expansion, as in plant cells. In some of Strasburger's figures (e. g. Taf. VII. Fig. 20) both ends of the spindle appear broadly truncate after the separation of the segments of the nuclear plate, and each truncate face occupying the centre of its aster is marked by a conspicuous structure in which the nuclear fibres terminate.

Following this condition is a stage in which the new nuclei are homogeneous. The latter never occupy the centre of the sun, but often rotate, as it were, about the former pole without reaching it. In this way two contemporaneously formed nuclei may eventually lie (as in Strasburger's Fig. 10, Taf. VIII.) farther apart than the centres of the two asters. In all animal cells which he has had the opportunity of studying, Strasburger finds that the new nucleus becomes at first homogeneous by the confluence of all the components of each half of the maternal nucleus (p. 226), that its definite formation beginning on the side toward the equatorial plane advances to the poles, and that the portion definitely perfected (equatorial portion) is distinguishable from the more homogeneous by its low power of refraction and by its nucleoli; further, that this equatorial portion has generally been taken for the whole nucleus. The nucleus may arise by the union of two closely situated vacuoles, but a greater number than two had never been seen. At the time its definite formation (Ausbildung) is completed, the nuclei are more or less pear-shaped, with the pointed ends occupying the centres of the asters. The rays disappear; the nucleus becomes rounded and a membrane is formed; it still continues to enlarge, probably taking its food from the surrounding homogeneous protoplasm (pp. 219 – 221).

When the products of the division cells are of unequal size (as in Unio), the "Mutterkern" takes an eccentric position, so that the plane of division is equidistant from the new nuclei. If one *side* of the spindle lie nearer the surface of the cell than the other, the constriction of the protoplasm begins earlier on that side of the cell.

Strasburger criticises O. Hertwig's description of the nuclear spindle as a band, since it is really cylindrical, and is shown to be so by Hertwig's own observations. He in turn admits the justice of Hertwig's criticism when the latter claims that the nuclei do *not* arise in Phallusia (as Strasburger first reported) at the centre of the asters. Both Auerbach and O. Hertwig have, he says, overlooked the homogeneous portion of the young nucleus, which reaches to the centre of the sun.* The interzonal filaments, like the nuclei, are susceptible of being nourished, and are thereby often considerably increased in mass and numbers, especially in the equatorial plane.

The general and theoretical considerations announced by Strasburger will be considered in connection with the review of his studies on plant cells.

Zeller ('76, p. 258, Taf. XVIII. Figs. 26 – 31) describes for Polystomum integerrimum remarkable changes in the nuclei during segmentation. After the two nuclear structures (pronuclei) have united, the resulting body disappears, and at two opposite poles of the egg there arise near the surface two clusters of vesicular nuclei with nucleoli. These nuclei increase in number and in size. Approaching each other closely, the components of each cluster are at length dissolved into a homogeneous mass. The cell now changes its form, elongating in the direction of the axis which joins the two clear poles, and finally from the more pointed end there grows out a bud which is ultimately pinched off. The single cell is thus divided into two cells of unequal size.

The most natural interpretation to be given the clusters of nuclei is that they are the same as the clusters seen by Bütschli and other observers to result from the metamorphosis of the "nuclear plate" of a spindle, although nothing like a spindle figure was seen by Zeller.

* In Limax I have seen the nucleus deviate considerably from the spherical form, and appear as though drawn out *toward* the centre of the aster (Fig. 80[a], compare also the pronuclei, Fig. 68) ; but there is nothing which gives support to Strasburger's view that a homogeneous portion of the nucleus reaches to the centre of the sun. I am even inclined to think that Strasburger may have fallen into an error in the case of Unio, by mistaking granules at the centre of the sun for evidence of a continuation of the outline of the nucleus to that point.

Their great number and enormous size as compared with that of the cell, their nearness to the surface, and their diffuse arrangement, combine to make this an interesting case of nuclear reconstruction.

Ed. van Beneden ('**76**^{d}, pp. 38, 47–52) discovered in one of the two forms of the parasitic Dicyema certain spherical striate bodies. These were always found in the vicinity of the germarium (germigène), and in size were, when occurring singly, like the germs. These were for a time thought to be spermatophores, but the author's attention was at length directed to their similarity to the nuclei of dividing cells as described by Bütschli and Strasburger. They were ultimately found to be germ cells in process of division. Immediately before dividing, says Van Beneden, the germ (a single cell) becomes very granular and opaque; the nucleus increases considerably in size, its contents lose much of their transparency, and its nucleolus disappears; then an extremely clear, meridional striation is developed at the surface of the nucleus; the striæ are not the result of an alignment of corpuscles or granules, but are due to the presence of continuous, homogeneous fibrils, formed of a very refringent substance. If one of its poles is directed toward the observer, this striation appears radial. The volume of the nucleus increases at the expense of the surrounding protoplasm to such an extent that the latter is reduced to a thin layer of granular substance enveloping the former. There soon appears at each pole of the nucleus a refringent "corpuscle polaire," around which are grouped very fine granules. The two poles become differentiated into granular polar disks, in which the ends of the meridional fibres are lost. At this time a polar view shows that the radial striæ are somewhat curved (Pl. I. Fig. 28, Pl. III. Fig. 3), — much as the *vitelline* rays are in Limax (Fig. 56), — proof that the fibres do not exactly follow the direction of meridional lines, but are a little oblique. The fibres exist only at the surface of the nucleus. The polar disks become thicker, more refringent, and distinct; the fibrils less clear, as if their substance were attracted toward the poles. The author has but rarely met with fibrils a little thicker in the middle than elsewhere; aside from this, he has seen no indication of either equatorial or lateral nuclear plates. Later, the polar disks are in some way condensed into small, discoidal, refringent bodies (pronucleus dérivé), around each of which is accumulated in the protoplasmic body a clear substance from which radial [extra-nuclear] striæ are sometimes seen to diverge (pronucleus engendré). The Zellplatte of Strasburger appears in the equator as a dark granular plate; a circular furrow appears at the equator of the cell; the cell-plate divides into two; the two hemispherical cells

remain in contact by that part of their surfaces which is developed by the division of the cell-plate. The "pronucleus dérivé" increases by blending with the "pronucleus engendré." The part of the old nucleus which remains striate and non-granular continues adherent to the cell-plate. The young nucleus enlarges, becomes clearer and more central in position, its contour regular, and it acquires a small nucleolus. Finally the last trace of the striated part of the old nucleus disappears.

RABL (**'76**, p. 318) often observed a karyolitic figure, especially in eggs (Unio) hardened by chromic acid, but is unable to give any account of the nuclear division itself.

In BOBRETZKY'S "Studies on the Embryonic Development of Gasteropoda" (**'76**), some attention is given to the changes of the nucleus during segmentation, especially in the case of Nassa mutabilis, Lam. (pp. 97–102). Usually the nucleus is not to be found after the egg is laid, but in one instance, by employing pressure, it became visible immediately under the surface of an egg which had already given rise to polar globules. Its contents had a homogeneous, water-clear appearance. Subsequently, there is a lengthening of the egg in the direction of the diameter passing through the formative pole, and, following this, a constriction in a plane at right angles to this diameter. At this time no nucleus is discoverable in either half of the incompletely segmented egg. Thin sections through the upper (or formative) half of specimens hardened in chromic acid exhibit the radial figures seen by Fol and others. In the middle of the finely granular substance are to be seen two clear spots without granules, from which there diverge toward the periphery rays which are formed of granules abutting upon each other to form straight lines. Those of the rays which are directed from the two centres toward each other unite midway between the spots, and such as lie on either side of a line uniting the latter are more or less curved. In the middle this system of curved lines is interrupted by a very narrow streak of highly lustrous granules, which are somewhat coarser than those composing the balance of the lines. This is Bütschli's spindle and its equatorial zone. Although on sections, the spindle stands forth somewhat more sharply than the radial rays, and is a little more intensely stained in indigo-carmine, yet it is of quite the same nature. Not only is there no nuclear membrane, as Bütschli thinks, but, says Bobretzky, "I cannot distinguish in it [spindle] the actual fibres, which, just like the rays, are nothing else than serially arranged granules." The appearance of two radial centres precedes the origin of the spindle-shaped body. In later stages of segmentation the two stars were already to be seen when the

nuclei were still *almost* unchanged (see also *op. cit.*, p. 108, Fig. 29 B. *n*). The author thinks he has seen, on sections of an egg presenting the first traces of the [first or] transverse furrow, the disappearing nucleus in the shape of a pale, hardly visible body midway between the two stars. It is described as an elongated, roundish, clear space, which differs only very slightly in its optical properties from the surrounding protoplasm, and is especially distinguishable only by the fact that the rays of the two stars which traverse it are interrupted at its boundaries. The limits of this space are thus clearly indicated by granules somewhat larger than those which compose the rays (Fig. 24).

It is with reluctance that one ventures to impeach the accuracy of another's observations, especially if the observer is one of such broad experience as Bobretzky; and yet I am convinced that his "disappearing nucleus" is in reality nothing more or less than the "nuclear disk" seen *obliquely*, — not edgewise, as in Fig. 23. The means of settling the question lay of course in the hands of the observer, for the two sides of the oval body could not have occupied the same niveau, if my interpretation is correct, and I confess it seems difficult to understand how this could have been overlooked by so competent an observer. It becomes all the more difficult to understand when, further on,* Bobretzky says that possibly the refractive granules surrounding the nucleus may be coördinated with the transverse granular band seen in the preceding figure. That the two are really the same structures, I have not the least doubt; but the author's attempt to explain their relation to each other cannot be regarded as successful. It is in accordance with an erroneous view of the origin of the median zone that he would interpret the meaning of this figure in case he is compelled to grant that its surface granules are identical with those of the transverse granular band. In that event, he says, one would be justified in drawing the conclusion that — in direct opposition to Bütschli's view — the granular zone (Fig. 23) is formed out of two rows of granules, which *approach each other* and unite in the middle of the [in the mean time] dissolved nucleus. That such an *approach* of granules takes place is based on the assumption that the egg drawn in Fig. 24 is less advanced (younger) than that drawn in Fig. 23, for a direct observation of the approach is not claimed. This as-

* ". . . . vielleicht wäre es möglich, die an den Grenzen des Kernes befindlichen glänzenden Körner mit dem queren Körnerstreifen der vorigen Figur gleichzustellen, woraus man den Schluss ziehen dürfte, dass die aequatoriale Körnerzone sich, der Meinung Bütschli's ganz entgegengesetzt, aus zwei, sich einander nähernden und in der Mitte des aufgelösten Kerns zusammentretenden Körnerreihen bildete." (p. 100.)

sumption in turn is supported by only two statements: the presence of the supposed nucleus in Fig. 24, and the slight development of the transverse constriction which affects the first segmentation (Fig. 24. *f*).

The appearance which the author has interpreted as a *nucleus* admits, as has just been suggested, quite a different explanation, which I shall directly attempt to strengthen by further evidence. As regards the second argument, — the shallowness of the constriction, — a comparison of Figs. 23 and 24 does not, owing to the incompleteness of the outlines, give satisfactory proof of the author's position; but a comparison of Fig. 1 (the egg from which the section shown in Fig. 23 was made) with Fig. 24 certainly leaves the impression that the constriction is, as the author claims, less advanced in the latter than in the former case, and that consequently the egg seen in Fig. 24 is probably younger than that shown in Fig. 23. I am, however, more inclined to agree with Bobretzky as to the relative advancement of the two eggs, on account of evidence which he does not seem to have given special consideration. I refer to the fact that in Fig. 23 the granular zone appears to be composed of halves which are separated by an appreciable interval, while in Fig. 24 the zone appears (according to my interpretation of the figure) still undivided. Granting that Fig. 23 represents a more advanced egg than Fig. 24, it by no means follows that the mutual *approach* of the rows of granules is the only possible explanation of the phenomena. Not only is it *possible* to refer the appearance to the obliquity of the granular zone, but I think a careful examination and comparison of the figures will show that this latter is much the more *probable* interpretation. Assuming that such a spindle-shaped body were viewed, not perpendicularly to the axis, but obliquely, a shortening of its *apparent length* would result. That is what is found to be the case in comparing the spindles in the two figures cited, that of Fig. 24 being *shorter* than that of Fig. 23 by about one tenth the length of the spindle. If, in answer to this, it were objected that the anterior segment of the egg itself is also somewhat smaller in Fig. 24 than in Fig. 23, and that consequently the shortness of the spindle is the result of the diminutive size of one of the eggs, I would suggest in reply, —

(1.) That there is a greater proportionate reduction in the length of the spindle than in the width of the egg;

(2.) That the diminished width of the anterior end of the egg might also be produced by the same obliquity; for it is reasonable to suppose, from what is known of the shape of other eggs at this stage of develop-

ment, that an elongation of the blastomere itself has taken place in the direction of the axis of the spindle; and

(3.) That both spindles have the same *thickness* at the middle, (the apparent thickness being unchanged by the supposed obliquity,) which would not be expected if the spindles differed considerably in size.

Although the observations made on Limax add another case to those where the stellate figures certainly arise before the disappearance of the nucleus, I think it will be difficult to show any case which shall justify the conclusions drawn by Bobretzky from his studies on the origin of the spindle; certainly his own figures do not warrant him in saying: "Obschon der Kern wenigstens in seiner äusseren Form noch unverändert erscheint, kann man schon hier den sogenannten spindelförmigen Körper unterscheiden, dessen Entstehung also keineswegs auf eine Umwandlung des Kerns zurückzuführen ist."

I must especially insist upon the insufficiency of Bobretzky's observations to establish this assertion. The statement is evidently based on such stages as those shown in Figs. 24 and 29. *n*. I have given above what seems to me a more reasonable interpretation of his Fig. 24, and have only to add that Fig. 29. *n*, although showing at the same time the nucleus with a nucleolus and the two stellate figures, does not exhibit the least trace of interstellate rays, — a spindle, — so that it does not afford any ground for the assertion, that "the origin of the spindle-shaped body is in no way referable to a metamorphosis of the nucleus."

Respecting the new nuclei, Bobretzky states, that they are to be discovered as small bodies connected by fine granular lines as soon as the segmentation furrow, which is to separate their respective cells, makes its appearance (Fig. 25); that subsequently the nuclei have become larger and the nuclear commissure has become almost invisible (Fig. 26). According to this latter figure the commissure is more conspicuous in the middle than near the nuclei, just as I have found it to be in Limax, except that the striate appearance is not at all represented in Bobretzky's figures.

Of the method in which the nuclei are formed, the author is not able to say anything very definite. In the stages just mentioned, he believes he has seen in the clear central spot of the stars an accumulation of very small pale vesicles, from which one might deduce the formation of new nuclei. No reconciliation of this untenable assumption with the accurate observation recorded in the following sentence is attempted. Bobretzky saw, namely, in one case clearly (Fig. 29 *B. m*) two new nuclei in addi-

tion to the stellate figures, and that they did not occupy the centres of those figures, but lay somewhat nearer each other (p. 108).

After careful examination the author comes to the conclusion that the large nutritive segment is destitute of a nucleus, and therefore refuses to acknowledge that it is a cell. It is only a detached portion of the nutritive yolk.

The final paper from Bütschli ('76), portions of the substance of which had already been made public in the two preliminary communications that have been passed in review, embraces a wide field of observation, and presents important additional information upon the phenomena connected with cell division.

In the case of Nephelis (p. 219) the stellate figures about the poles of the spindle receive an attention not accorded them in the preliminary papers. Around each of the ends of the first spindle (Richtungsspindel) is to be seen a clear area (Hof), distinguishable from the remaining yolk mass by its homogeneous condition, from which the yolk granules stretch out radially through the yolk in all directions, — "ein Strahlensystem oder eine Sonne." The clear area possesses no definite boundary toward the granular yolk, but merges gradually into it (p. 216).

The nucleus of the first segmentation sphere exhibits a distinct, dark envelope (Hülle), and embraces no nucleoli, but instead a clear fluid which is traversed by a number of protoplasmic cords which enclose here and there dark refractive granules, and which are often united into a network. The first segmentation is introduced by an elongation of the yolk and the metamorphosis of the nucleus into a spindle. At each of the opposite points of the nucleus which fall in the axis of elongation, there arises in neighboring parts of the yolk a radiation, and at once there begins to appear in the centre of each a clear area of the kind just described. Between these two points the nucleus now begins to undergo a longitudinally fibrous differentiation. While this differentiation advances, the still unaltered nuclear remnant continues to exhibit, though less distinctly, its previously described structure, till it at length completely disappears. The volume of such a spindle-shaped metamorphosed nucleus is less than that of the original. The change, in Bütschli's opinion, can only be explained by supposing that a portion of the fluid of the nucleus escapes during the metamorphosis.

I pass over points already reviewed in the preliminary papers, and only add that Bütschli saw the fibres of the Kernspindel again become thickened and darker in the equator after the beginning of the segmentation, and thus form the so-called cell plate of Strasburger (p. 219).

The metamorphosis of the primary segmentation nucleus into a spindle was not so satisfactorily traced in the case of Cucullanus elegans. Possibly a stage in this change is represented, says the author, in Fig. 20, where, in place of a nucleus, there is only an indistinctly defined clear spot in the centre of the yolk, within which spot a number of dark granular rods are irregularly disposed. This and all subsequent spindles differ from the "Richtungsspindel" in that the rods of the nuclear plate in the former lie within a definitely limited body, and cannot therefore be simply a differentiation in the yolk (p. 224), while in the latter the nuclear plate is formed of only a circle of dark granules (p. 226).

When the new nuclei have made their appearance in the place of the lateral plates, the nuclear fibres are no longer to be seen. Each nucleus arises from a few (two to four) separate nuclei, which subsequently unite. A distinct nucleolus is found only at a much later stage. The radial structure of the yolk is to be seen during segmentation, as in other eggs, but on account of the extremely fine-granular nature of the yolk it is relatively difficult of observation.

In his studies on the gasteropods Limnæus and Succinea, one looks for a close agreement with the phenomena which take place in Limax. The division of the primary segmentation nucleus begins in the still spherical yolk by the appearance of two small radial systems at diametrically opposite points of the nucleus, which determine the axis of the subsequent division.

In Bütschli's Fig. 10, Taf. IV., which is here cited, one sees that the stellate figures arise at points on the nucleus which are not, strictly speaking, *diametrically* opposite, but rather somewhat nearer the centre of the egg than is the centre of the nucleus; just as we have seen in Limax that the asters are somewhat deeper than the centre of the two pronuclear structures taken as a whole. The centre of each star is occupied by a homogeneous clear area. Since acetic acid was used by Bütschli, it is not strange that a central, more refringent structure was overlooked. Other reagents would doubtless have disclosed the fact that this area is not entirely homogeneous. In a subsequent stage (Fig. 11) the nucleus has assumed a streaked appearance. At first the dark interior corpuscles of the nucleus are visible between the streaks, but they soon disappear, and the nucleus becomes a longitudinally striate spindle, stretching between the two suns. Bütschli has figured (Fig. 13) an egg in which the constriction of the yolk is conspicuously advanced, and in the middle of the spindle are seen fibre-thickenings, which he considers to be the *nuclear* plate. Although it is hardly safe to infer from a comparison

with observations on Limax that these thickenings represent the *cell* plate, and that the young nuclei have been overlooked, nevertheless, if this is not the case, there must evidently be a want of synchronism in the events of cleavage in the eggs of these two gasteropods, for at a nearly corresponding stage of segmentation in Limax (Fig. 90) the halves of the nuclear plate are already far apart. That, however, which seems to render the first supposition almost certain, is the fact that the testimony of other observers who have carefully studied these stages leads to the same conclusion. I will call attention to the statements of Hertwig (**'75**, pp. 409, 410, Fig. 25) and Bobretzky (**'76**, p. 101, Fig. 25), and to the fact that Bütschli's own figures of Succinea (Taf. IV. Fig. 19), Cucullanus (Taf. III. Fig. 21), and Nephelis (Taf. I. Fig. 12) point to the same conclusion. It is to be noticed further, in regard to the last-mentioned figure and its explanation, that the author places himself in an ambiguous position, unless "*Kern*platte," in the statement, "Die sogennante Kernplatte ist gebildet," is a misprint for *Zell*platte.*

The author informs us that he has not observed the separation of the halves of the nuclear plate, from which I conclude that he has not observed any stages showing the halves of the plate, for he has given no such figures, and a *direct observation* of the migration is hardly to be thought of in such opaque eggs. The new nuclei are also in this case, without doubt, formed by a differentiation of the halves of the nuclear plate which have migrated into the ends of the spindle. Where they have already appeared (Taf. IV. Figs. 14, 19) one sees clearly that the fibres which join them are again swollen in the middle and have become dark and lustrous. I am at a loss to understand the figures given by Bütschli in this connection. They correspond in no way, as far as regards the shape and appearance of the *thickened* portions, with anything I have seen. If the fibres of the lower half of the spindle represented in Fig. 19 were slightly thickened in the middle, and the upper half were entirely absent, the figure would very closely correspond to what I have many times seen in Limax. The nuclei increase in size and remain united by the interzonal filaments when the segmentation is otherwise completed. The author did not observe what became of the cell plate.

The phenomena presented by Rotifera agree very well with the obser-

* Since writing the above I observe what had escaped my notice before, — that Bütschli has expressed, in his explanations of Taf. IV. Fig. 13, a doubt as to this structure being after all a *nuclear* plate. It seems to me that, with the material at command, he might have expressed himself even more decidedly in favor of its being a *cell* plate.

vations on Nephelis. In Brachionus (p. 247) the radial figures appear suddenly in the yolk at two opposite points of the nucleus, and at each of these places is formed a re-entrant surface. These surfaces advance till they meet, and thus seem to cause the disappearance of the nucleus. The process, however, as acetic acid preparations show, is only a nuclear *metamorphosis*, which advances from the points mentioned. The division of the nuclear plate and the migration of its halves was observed in Notommata. In the two genera mentioned, only a single new nucleus is formed in each new segment. Why Bütschli cites in this connection Flemming's ('**75**, Taf. III. Fig. 2) figure of *Anodonta* as making probable the ultimate discovery of a cell plate in the *Rotifera*, I cannot conjecture, unless he made the mistake of supposing that the figure above cited was that of a rotifer's egg. Bütschli remarks, with reason, that he has never, even in the rotifers, seen the new nuclei in the centre of the stellate figure, as Flemming has drawn them in the case in question.

In a pseudovum of Aphis, Bütschli also once saw two small nuclei joined by delicate filaments. The nuclei of the blastoderm, he therefore concludes, arise by successive divisions of a single nucleus (p. 249). Moreover, the blastoderm cells of a butterfly and of Musca vomitoria (p. 261) were demonstrated to present the striate spindle-shaped differentiation of the nucleus. In the former the equatorial nuclear plate was clearly seen; in the latter, only irregularly distributed local thickenings of the spindle fibres. In Musca the radiation of the protoplasm about the ends of the spindle was very distinct.

The second section of the fourth chapter of Bütschli's paper (pp. 394–419) is devoted to a general consideration of nuclear and cell division. The author considers, with Strasburger, that the increase of nuclei by means of a metamorphosis into a fibrous spindle-shaped structure is to be regarded as the original and typical method. But there exists without doubt another mode of nuclear division which greatly differs from this, or at least may be referred back to it only by assuming very radical modifications (e. g. blood-disks of Rana, etc.). Supported by the existence of a very delicate, yet exceedingly distinct membrane, enveloping the nuclear spindle of the infusorian "nucleoli," the author assumes a similar membrane for all other nuclear spindles, and as evidence of his correctness calls attention to the distinct contour which was seen by Strasburger to surround the nuclear plate of certain vegetable cells when the plate was viewed *en face*. The indistinctness of the nucleus in the living egg when it is undergoing its spindle metamorphosis the author explains as due to three causes: (1.) the disappearance of the so-called

nuclear membrane, whereby the nucleus loses its sharp limitation from the protoplasm; (2.) a uniform distribution of the nuclear substance through the whole nucleus; (3.) a loss of clearness. This last is attributable to a loss of nuclear fluid during the metamorphosis, which may in the observed cases of Cucullanus and Nephelis amount to one third, or even two thirds, the volume of the unaltered nucleus. What becomes of this fluid (wässeriger Kernsaft)? It is not uniformly appropriated, says Bütschli, by the surrounding protoplasm; on the contrary, it may be inferred — from the fact that the metamorphosis of the nucleus begins at two points, and that each of the neighboring radial systems of the yolk embraces a clear homogeneous central area, which is at first small, and increases with the metamorphosis of the nucleus — that this fluid escaping at these two points, as Auerbach maintains, becomes accumulated in the central areas mentioned. So crude a conception of the origin of the radial figures as Auerbach entertains cannot, he says, be accepted, nor yet can Flemming be right in considering them due to a *structural condition* of the protoplasm. First of all, the seat of the cause of the radial phenomenon is to be sought in the central area. The latter, however, does not correspond to the end of the nucleus, and for this reason Strasburger is at fault in referring the cause to an attraction which operates upon the surrounding protoplasm from the ends of the nucleus. For the same reason the area itself cannot be regarded as a mass attracted by the end of the nucleus. What may be the nature of the changes produced in the protoplasm of the area by the nuclear fluid, he is unable to say; perhaps it is only a simple swelling and solution resulting in the homogeneous and light appearance of the area.

Aside from Bütschli's failure to see a central structure in the area, it seems to me that the optical properties of the latter — it being more highly refractive than either the surrounding protoplasm or the nuclear fluid — are not easily reconcilable with this interpretation.

The only deviation from a fusion, sooner or later, of the elements of the nuclear plates, is that observed in the case of the primary nuclei of Infusoria during *conjugation.* In the case of Paramecia, at least, the plates retain their differentiated condition so that the fibrous, spindle-shaped nucleus divides into two, each of which has a structure like the original.

Although a trace of a cell plate is to be found in Nephelis and snails, it is unlike that found in plants, for there is no spreading out of the nuclear fibres and the plate does not take part in the formation of the cortical layer (Hautschicht) of the segments.

It is admitted concerning the formation of the daughter nuclei out of the halves of the nuclear plate, that in segmentation spheres the fusion of the plate elements has not been established with certainty. The conclusion is reached, however, that the homogeneous and compacted condition is the original and simplest form in which the nucleus appears, quite contrary to Auerbach's notion of a *fluid cavity* in the protoplasm. So, too, the nuclear membrane is not produced from the protoplasm surrounding the nucleus, as Auerbach maintains, but, like the nucleolar structures (Binnenkörper), is a differential product of an originally homogeneous corpuscle. In Cucullanus, in Nephelis, and possibly in snails, there are differentiated out of the nuclear plates at first several small nuclei instead of one, each of which afterwards becomes for itself differentiated into a vesicular nucleus, as does in other cases the single nucleus.

This conclusion arises in part from Bütschli's failure to recognize the nature of one of the nuclear structures, the male pronucleus. But aside from that, I have only to say that I have seen nothing of a like nature in the case of Limax.

Much evidence of similar nuclear conditions is accumulated by Bütschli at pages 409–412, in the course of which he mentions having seen very distinctly in sections through eggs of Rana temporaria the radial structure of the protoplasm around the halves of the "Lebenskeime" (Götte), when the latter had already advanced into the daughter cells, — a phenomenon which escaped Götte's attention.

The more a daughter nucleus grows, the more the central area of the neighboring radial system diminishes, and the former gradually advances to the position of the latter, whence it is to be inferred that the area furnishes the material for the growth of the nucleus; this consists of fluid, and also of genuine nuclear substance. The nucleus acquires, however, some of this nuclear substance by the retraction of the interzonal filaments. When the growth of the daughter nucleus ceases, the central area and the radiation have totally disappeared.

The want of precision, already noticed, in the account of the time when the segmentation furrow appears, is, if possible, emphasized by the author's saying that the first trace of the division of the yolk appears "somewhere about the time" of the division of the nuclear plate and the separation of its halves, — a period, I should say, of rather indefinite duration, notwithstanding the rapidity with which the halves of the plate begin to move asunder.

An important *rôle* in cell division is to be ascribed, says Bütschli, to

the nucleus; in many cases it is the immediate cause of the division, and it is no contradiction of this that nuclear division may take place without an accompanying cell division, for the field of its (nucleus) influence must have a limit. Most clearly does the nucleus sustain a causal relation to the division in those cases where it is eccentric in position, inasmuch as in these cases the segmentation invariably begins on the surface of the yolk nearest to the nucleus.

The idea that the radial systems are due to centres of attraction, as the author, together with others, once maintained, he is now inclined to surrender, because it leaves the cause of the yolk division quite unexplained. He now maintains "that the radial arrangement of the plasm around the central area is the expression of a physico-chemical alteration of the plasm, emanating from the area, and that a gradual diminution of this alteration — which receives its support from the central area — takes place from the central area toward the periphery."

The assumption that the radial structure is of this nature is, in Bütschli's opinion, sufficient to account for the origin of an *inequality* in the superficial tension of the sphere in case the chemical changes tend to an *increase* of tension.* This tension will necessarily be restored to equilibrium in accordance with the physical law that the superficial tension is inversely proportional to the radius of curvature. As the maximum tension will occur where the activities of the two centres combine (viz. in the equator †), the equilibrium will only then be restored when the radius of curvature at the equator is increased, and at the poles is diminished. But this is equivalent to a prolation of the sphere. The chemical changes continue, and as the prolation results in removing the poles of the spheroid farther from their respective central areas, and in bringing the latter nearer to the equator, the difference in superficial tension must become exalted. A constant increase in the superficial tension at the equator will result finally in an "Einfurchung" in that plane, inasmuch as by this means the force of cohesion will be considerably diminished by its outwardly directed negative component.

Aside from the author's confessed inability to explain fully the subsequent "Durchfurchung," one feels impelled to inquire what ground

* The sphere obeys the fundamental laws of a fluid mass.

† The author admits that this plane, as is evident, will be the equator only under certain conditions relative to the rapidity with which the changes in the plasm are propagated, but thinks that these conditions may well be assumed to be present. On the other hand, it may be said that in Limax the stellate figures are certainly not always synchronous in their origin, — that one may have attained considerable dimensions before the other is discernible.

there is for the assumption that the physico-chemical changes result in an *increase* rather than in a decrease of the superficial tension.

The author locates the causes of the metamorphosis of the nucleus which finally lead to its division in the surrounding protoplasm, as Auerbach maintains, rather than in the nucleus itself. An evidence of this is to be found in the fact that, if several dividing nuclei occupy the same protoplasmic mass (Infusoria, etc.), they are found to present the same stage of advancement. This is, in his opinion, explainable by assuming that the protoplasm acts alike and simultaneously on all the nuclei.

Although agreeing with Bütschli as to the location of the force which induces nuclear division, I am not able to rest my belief on the evidence which he brings forward here. I know no reason why one would not be equally justified in explaining the synchronism in the division of the several nuclei by assuming that all the masses of nuclear substance are subject to the same laws and rate of growth, — that each requires the same time in preparation for division. The *synchronism* of the events is no more an argument in favor of an initiative activity on the part of the protoplasm than on the part of the nuclear substance. There may be no objection to saying that the protoplasm acts alike and simultaneously on all the nuclei; but it might be said, with equal justice, that all the nuclei, being chemically alike, act alike upon the protoplasm, and, being of the same age, act simultaneously.

In his paper on the development of Heteropods, Fol (**'76**, pp. 112–114) still maintains opinions already alluded to in connection with his paper on Pteropods. Not only does the nucleus disappear at each segmentation, but it becomes twice fused with the surrounding protoplasm, and as many times individualized, before the first segmentation (p. 113). The nucleus which has reappeared in the central star disappears again to give place to two centres of attraction, etc. "The segmentation takes place in the well-known manner. Then the nuclei reappear in the centres of attraction of the first two spherules, and the same phenomena are reproduced at each of the subsequent segmentations." (p. 114.) The nature of the spindle may be learned from the account given of the maturation spindle. "The stoutest of these (aster) rays are those which pass from one centre to the other in the interior of the nucleus." The fusiform body (Auerbach) he considers to be only the central part of the disappeared nucleus; it is the body described in Geryonia as the remnant of a nucleus. As to the fibres, they are only striæ in the protoplasm (p. 112).

In his "comparisons et reflexions," the author treats these phe-

nomena with such freedom of interpretation that one is constantly meeting with surprises. He has maintained, as above stated, that the nucleus becomes fused with the surrounding protoplasm, but now claims (p. 138) that its disappearance is not caused by a veritable dissolution and mixing of its substance with this protoplasm, but that it is due rather to a molecular change which renders it optically like the protoplasm, but without dispersion of its elements; and presently (p. 139), when speaking of Bütschli's opinions, he says: "Je crois volontiers que le nucléus se partage en deux et que cette division est visible chez les vers."

It appears to me another question whether, in its division, the nucleus has an active *rôle.* Certainly there is much to show that the activity is not all on its part. As the author justly remarks, it cannot serve as the centre of attraction presiding over the division of the cell, since the centres of attraction originate at the boundary of the nucleus and protoplasm. Although I cannot unreservedly subscribe to the belief that the successive modifications of the nucleus take place in a manner "tout à fait *passive*" for it, it is much easier to indorse the statement that the nucleus deports itself in a manner quite as passive as the rest of the cell.

Fol's writings up to this time teach unequivocally that the new nuclei arise *at the centre* of the radial structures of the protoplasm; it is therefore surprising that the author now expresses his assent to Auerbach's discovery,* as though there were no fundamental difference between them to be explained, and then calls attention to the grave error of Strasburger, who mistook the protoplasmic mass (area) surrounding the centres of attraction for the nuclei.

Bütschli's spindle fibres are, in Fol's opinion, filaments of sarcode; the granules (of the nuclear plates) are varicosities of the filaments, which have no relation whatever with the nucleoli.†

Fol states, a little farther on, that the substance of the old nucleus appears to contribute to the formation of the new nuclei.

Stossich ('76) has observed the radial arrangement of the protoplasm

* "Du reste, Auerbach a remarqué avec justesse que les deux taches claires qui paraissent représenter le nucléus divisé et momentarément modifie, reparaissent dans une position excentrique et se rapprochent ensuite du centre de chacune des deux étoiles moléculaires."

† The author seems to have made a slight mistake in stating (p. 141) that Bütschli's first accurate description of the spindle and its fibres is to be found in his communication in the July (1875) Heft of the Zeitschr. f. w. Zool. A very good description will be found in the March number of that periodical, p. 208.

in Serpula immediately preceding and accompanying segmentation. (See p. 428.)

After alluding many times to the priority of his own discoveries in this field of research, Fol ('76[a]) communicates important new considerations resulting from his studies on Heteropods, the sea-urchin, and Sagitta.

The centres of attraction appear before each segmentation at opposite poles of the nucleus, which is still absolutely intact, and seem to be local fusions of the substance of the nucleus [not quite "absolutely intact"?] with the vitelline protoplasm, or perhaps an irruption of protoplasm into the more fluid interior of the nucleus.

The difference in the appearance of the intranuclear (spindle fibres) and the extranuclear filaments results from the former being immersed in a medium almost liquid and much less refringent than the protoplasm of the filaments, while the latter are bathed in protoplasm, and for that reason ought not to be so easily distinguishable. The difference between these filaments is therefore only apparent and depends on the properties of the substances surrounding them.

If the varicosities discovered by Bütschli appeared only upon the intranuclear filaments, they would, in Fol's opinion, establish a remarkable difference between the two kinds. But that is not the case. In the eggs of Geryonia and the sea-urchin varicosities are to be found upon the extranuclear filaments, which have hitherto escaped all observers. These enlargements are more elongated and less regular than those of the interior of the nucleus, but they are, after all, indubitable varicosities, which migrate like the others, and slowly become fused with the central mass of protoplasm. This mass is, therefore, neither in its mode of origin nor growth exclusively a derivative from the substance of the old nucleus; it is the result of a fusion of a part of this substance with a part of the vitelline protoplasm.

As to the relation of these central masses to the new nuclei, the author says he has often observed that, after having absorbed the greater part of the radial filaments and their thickenings, they exhibit spots which are clearer and probably more liquid than the rest of the mass, and which have on this account been styled vacuoles. The new nucleus is the result of the fusion of these vacuoles. That which remains of the central mass constitutes the envelope of the nucleus. Often, *but not always*, a vacuole arises outside the central mass, on the side toward the old nucleus. This shows that the liquid of the nucleus has the same double origin as the masses themselves. The new nuclei result from a partial

liquefaction of these masses; they are therefore composed of a mixture — in very different proportions in different cases — of the substance of the old nucleus and the protoplasm of the cell.

The observations made on Limax confirm much that is described by Fol. I am more inclined to think the asters result from a fusion of some part of the nuclear contents with the vitelline protoplasm, and that it is the chemical activity thus brought about which induces the radial appearance, than to assent to so crude an explanation as is implied by an irruption of the protoplasm into the nucleus, however much the figure in Fol's paper on the Pteropods (Pl. VIII. Fig. 4) may resemble such an invasion.

While I do not believe the whole difference between intra- and extranuclear filaments can be ascribed to the media in which they are found, — which, according to Fol himself, become optically the same! (see above, p. 325), — the occurrence of thickenings in the extranuclear filaments is sufficient to suggest a closer relationship between them and the spindle fibres than has been generally admitted. My own studies are not adequate, I regret, to either confirm or refute the observation respecting the *migration* of the extranuclear varicosities.

The part of Fol's description which agrees least with my own observations is that which concerns the relation of the new nuclei to the central masses (areas) of protoplasm.

The second paper by O. Hertwig (**'77**) on "Bildung, Befruchtung und Theilung," etc. embraces studies on the eggs of two of the Hirudinea (Hæmopis and Nephelis), and of Rana temporaria and R. esculenta. Although this paper does not undertake the discussion of cell division and the accompanying changes of the nucleus, it indirectly has much to do with these phenomena, since the conclusion is here for the first time reached and formally presented, that *the production of the polar globules takes place in the manner of cell division.*

In a foot-note Hertwig (pp. 48, 49) says a few words about cell division in frogs' eggs. Soon after the division of the segmentation nucleus, there is found imbedded in the finely granular substance of each of the heads of the dumb-bell figure a group of numerous large and small vesicles, which are mutually flattened by reason of their closeness. These are tinged in carmine, and possess the properties of small vacuolar nuclei. They are produced from the separate granules of the condensation zone, which simultaneously imbibe nuclear fluid. Each group therefore corresponds to a *single* daughter nucleus.

The division of the yolk appears to be effected by the contraction of

its protoplasm, especially of the superficial layers. The pigment which is located in the latter follows the depressions of the furrows.

Of still more interest, since corroborating the observations made on Limax, is the brief statement made (p. 24) concerning the appearance of the spindle and the mutual relation of the conjugating pronuclei in the case of Nephelis. Hertwig has never seen these two structures melt together into a single nucleus, notwithstanding the examination of numerous preparations; not even in cocoons presenting at the same time eggs with conjugated nuclei and others about to undergo the first segmentation. He therefore concludes that this fusion stadium can probably be of only short duration. Perhaps the fusion in the majority of cases takes place only when the two flattened nuclei begin to elongate and become metamorphosed into a spindle.

It seems to me probable that Nephelis is not unlike Limax in this matter, and that the observer may in Nephelis look in vain for an elongation of the pronuclei, since in reality a fusion of these structures is an accomplished fact only when the nuclear spindle is formed. Hertwig's suggestion, that the fusion may take place synchronously with an elongation of the pronuclei, does not rest, as I understand, on the observation of an elongated condition, but is inferred to exist from analogy with cases where the two pronuclei fuse and the resultant segmentation nucleus elongates. I would suggest that a still more radical difference may obtain here, and that neither a distinct morphological segmentation nucleus nor an elongation of the separate pronuclei finds expression in the case of Nephelis.

Brandt's (**'77**) paper on segmentation in Ascaris aims at a reconciliation of the earlier observations of Bütschli (**'73**[a]) and Auerbach (**'74**), by calling especial attention to the amœboid nature of the germinative vesicle, and of nuclei in general. It is possible (p. 374) that the *extensive and ramified* pseudopodia of the germinative vesicle first segmentation nucleus], which at the time of segmentation are vigorously amœboid, exercise a strong stimulus upon the contractile yolk substance, and thus favor segmentation.

The neglect to use reagents explains the fact that Brandt failed to see anything of a spindle structure or its elements, and perhaps also that the radial phenomena which he observed were only such as unfavorable objects have revealed to other observers. The new nuclear structures which Auerbach describes as vacuoles in the handle of the dumb-bell are only an indication to Brandt, that the nuclear pseudopodia first to be retracted are those occupying the side of the cell next

the plane of segmentation. These, however, are not always the first to be withdrawn. The "vacuoles" may result from a mechanical stimulus inaugurated by the rupture of the nucleus. (!)

As segmentation advances, the cells become successively smaller, and the nuclei, though absolutely smaller, are proportionately much larger than at first. They grow at the expense of the yolk, which ultimately is *entirely* consumed, — a confirmation of the theory that the germinative vesicle is the primary egg *cell*.

The influence of abnormal fecundation on the process of cell division as given by Fol (**'77**^{b}) will be found in the part of the present paper devoted to fecundation.

McCrady (**'77**) seeks the explanation of segmentation in the amœboid nature of his "protembryo" (p. 177), and is compelled to assume the existence of an *unseen protoplasmic matrix* to explain, for example, the approach and mutual flattening of segmentation spheres after their separation. He finds himself compelled to admit that O. Hertwig's observations make it impossible to exclude a *selective polar force*, and he proceeds to show how this polar force may be supposed to operate in segmentation. Although apparently irreconcilable, McCrady thinks the two theories (Hertwig's and his own) may not be really incompatible.

Brandt (**'77**^{b}) has communicated observations on the eggs of Lymnæus stagnalis and Anodonta anatina, in which the amœboid nature of the germinative vesicle and of the nucleus is deemed sufficient to account for the phenomena accompanying maturation and division.

In the case of Lymnæus (pp. 591 – 593) the condition of the nucleus during the first part of the first segmentation was not satisfactorily followed, but during the stage of the mutual flattening of the segments the nuclei were seen. At this time they appeared as clear structures of a stellate shape, which were possessed of ramified, more or less radial pseudopodia, and were constantly undergoing changes of form. It was owing to the instability of their forms that they were at times very distinct and sharply defined; at other times less distinct, or even invisible.

After citing several authors whose observations are thought to be capable of an interpretation substantiating the amœboid hypothesis, the author says (p. 598): "Im Ganzen herrscht bei den citirten Autoren die Ansicht vor, die strahlenförmigen Figuren wären auf ein Structurverhältniss des Dotters zu beziehen. Meine früheren Beobachtungen am Ascaridenei zwangen mich dieser Ansicht entgegenzutreten und die Strahlen für Pseudopodien der Furchungskerne zu halten; zwischen ihnen müssen eo ipso auch die Dotterkörnchen sich strahlenförmig

lagern. Dieses Resultat bin ich nunmehr im Stande auf Limnæus auszudehnen, und so dürfte wohl der Nachweis ähnlicher Strahlen für die übrigen Thiere einen Rückschluss auf die amöboide Beweglichkeit der Furchungskerne gestatten."

The close relationship between Limax and Lymnæus allows me to speak with more confidence than might otherwise be the case. It is to be noticed that the phenomena which Brandt interprets as due to extensive amœboid movements of the nucleus, are thus explained on what seems to be very insufficient evidence. The inference drawn from the fact, that in place of distinct nuclei there are to be found only irregular and often indistinct figures which undergo change of form and position, is far from satisfactory. Further, proof is not produced that the irregular amœboid figures are nuclear structures. Nor is his position strengthened when it is subsequently maintained that the so-called nucleus is, in reality, the *cell;* for enough is known of this so-called nucleus to warrant the expectation that it will respond in a definite way to various reagents. These, however, the author does not seem to have employed, and it must be largely due to this fact, that his observations present with undue prominence certain features of cell activity and entirely ignore fundamental internal changes. If we both arrive at the same negative conclusion respecting the complete dissolution and disappearance of the germinative vesicle and its descendants (nuclei), it is nevertheless from quite different data. The *motion* which results from the amœboid character of the nucleus is not competent to explain its admitted absence (Brandt, **'77**^{b}, p. 592 and Fig. 7), but on the contrary should make the moving masses — the pseudopodia — more readily discernible. Even without the evidence which other methods of research bring to bear on this question, I should agree with Warneck (**'50**, p. 115), who has observed similar phenomena and referred them to an unequal distribution of the elementary corpuscles of the *yolk.* If it be objected that he makes no attempt to explain the *cause* of the unequal distribution of these corpuscles, and that consequently he may not be cited as conflicting with the amœboid theory, I reply that he does not anywhere admit such radical changes in the form of the nucleus as Brandt maintains, and that some of his figures appear to me quite inexplicable under that theory. I would especially call attention to his Tab. IV. Fig. 10″ (compare with the text at p. 125), where the inequalities in the distribution of the corpuscles are as conspicuously represented as in any of his figures, and where *the nuclei* (two pronuclei) *are represented with the greatest distinctness as sharply defined spheroidal bodies.*

I am strengthened in my belief that the phenomena described by Brandt are only more extensive exhibitions of what Warneck has depicted, by the fact that Brandt has himself cited the observations of the same author, and even called attention to Figs. 3′ – 5′ of the above-mentioned plate in confirmation of his theory.

Bischoff (**'77**), to whom Embryology owes so much, has recently contributed to the discussion of the questions under consideration, without, however, bringing new material to the elucidation of the subject.

Two points of very general interest receive his attention : — (1.) The unreliable methods of investigation, by means of hardening and staining reagents, etc., which have recently become so popular, are responsible for misunderstandings and misconceptions which otherwise would be avoidable. (2.) The greatest confusion as to the nature of the animal cell has arisen through a confounding of its *morphological* with its *physiological* nature. While all agree that the cell is physiologically an *elementary organism*, things the most diverse, from a *morphologico-histologic* standpoint, are indiscriminately called cells. The same confusion is responsible for the idea that the egg is a primary cell. "Meiner auf Erfahrung, so weit sie reicht, gebauten Ansicht nach, ist nur das Keimbläschen eine wahre primäre und die einzige Zelle, von der bei dem nicht in der Entwicklung begriffenen Ei die Rede sein kann, und das Ei selbst wird am passendsten ganz allgemein als eine Umhüllungs-Bildung einer Zelle aufgefasst. Ich glaube ferner, dass jedes *reife* Ei an seiner *primären Bildungsstätte im Eierstocke* nur, aber auch immer, aus Keimbläschen, Dotter und Dotterhaut besteht." (p. 12.)

As regards the fate of the nucleus during segmentation, although granting that the question has not reached a final elucidation, Bischoff (p. 43) evidently inclines to the side of Auerbach and those who believe in its dissolution at each act of division. Were Bischoff to write in the light of what has been described within the past two years, it may fairly be doubted if he would persist in saying that new nuclei are formed quite independently of the old nucleus; * there is already too much evidence, independent of the supposed deceptive appearances produced by reagents, to make this position longer tenable.

It seems incumbent on those who hold, with Bischoff, that the germinative vesicle is not a cell *nucleus*, to explain why the vesicle undergoes the same modifications previous to the formation of the polar globules

* "Bei der Einleitung zur ersten Theilung das Keimbläschen und sein Kern schwinden, und ein neuer Kern sich *unabhängig* von diesen bildet. Was das erstemal geschieht, wird auch wohl das zweite- und drittemal geschehen." The original is not Italicized.

that the nuclei of embryonic cells and of segmentation spheres present initiatory to the acts of division.

Two communications from Giard (**'76** and **'77**) are in so far of interest here as they ascribe to Bütschli the idea that the polar globules arise in the eggs of Lymnæus, Succinea, Nephelis, and Cucullanus by the process of cell division; the author then says that he is able to add that the same is the case with Salmacina Dysteri, and Spirorbis (**'77**, p. 566).

I do not know how the author can ascribe this idea to Bütschli, for the latter seems not to have yet arrived at that conclusion when the first paper (Giard, **'76**) was published, and gave no expression to such a notion until some time after Giard's second paper had appeared.

P. Mayer (**'77**, pp. 212, 213, Taf. XIII., XIV.) says that no nucleus is to be found, even with the use of the ordinary reagents, in *freshly* laid eggs of Pagurus Prideauxii, but in the course of a few hours one becomes visible. Subsequently the single nucleus has given place to two, then to four, and finally to eight. It is only after the formation of eight nuclei that the segmentation begins, nor are there eight cells formed at once, but first two, then four, then eight. Mayer did not, on account of the opacity of the eggs, observe the division of the nuclei. It is not possible to say from his figures whether the nuclei undergo a spindle metamorphsis, though the same is to be inferred, since he refers to the presence of Auerbach's karyolytic figure as a matter that scarcely needs to be stated. In his opinion, however, the nucleus is not dissolved, but is directly divided. He has seen long-drawn nuclei and even two nuclei in a cell about to divide, without thinking it necessary to conclude that they are pathological conditions.

Stossich (**'77**) gives an account of the nucleus during division, which will be noticed elsewhere (p. 448).

Bütschli (**'77**^{b}, p. 236) states that he has, at the same time with, and independently of Giard, arrived at the same conclusion touching the cell nature of the polar globules, though he lays no claim to having expressed that opinion in the works which were accessible to Giard when he wrote. "Giard hat sich auf Grund meiner früheren Beobachtungen diese Ansicht gebildet."

This paper of Bütschli contains no further contribution to the nature of cell division and nuclear changes, save the exception which is taken (p. 240) to Robin's view, — that smaller segments arise from larger ones in the case of Nephelis by a budding process, in which the nucleus takes no part. Robin's position is sufficiently refuted by Bütschli's figures and remarks.

Hoffmann ('77) was unable to follow any of the internal changes accompanying the segmentation of eggs of Malacobdella (p. 23), but found that a constant change of the external form of the yolk accompanies the earlier segmentations. In Clepsine the same author ('77[a]) has by means of sections been able to see something of the stellate figures (of which his Fig. 10 gives a rather diagrammatic view), but nothing of a spindle figure during segmentation (p. 35).

Selenka ('78) has given a preliminary account of observations on living eggs of Toxopneustes variegatus.

During the union of the sperm- with the egg-nucleus to form the primary cleavage nucleus, the plasma, which constituted the central area of the radial figure surrounding the former, in part flows around the egg-nucleus, and thus gives rise to a second central area. These two central areas diverge, the cleavage nucleus becomes ellipsoidal and suffers a metamorphosis, while the yolk becomes flattened in the direction of the axis of the nucleus. The latter exhibits at each pole one, and then several deep incisions, resulting in a longitudinal fission of its whole substance into some twelve cylinders (Kerncylinder). An equatorial nuclear plate arises and splits into two plates, each composed of twelve "Kernstäbchen," or "Vorkerne," arranged in a circle. These migrate to the respective ends of the nucleus, which has meantime become an elongated cylinder, and here they melt together, first into six, then into two, and finally, but slowly, into a single new nucleus, which *rapidly* grows to double the size it had at first. A constant increase in the mass of the "Vorkerne" accompanies their union, and is caused by the absorption of the contents of the "Kerncylinder," which is really composed of processes of the "Vorkerne." The flattening of the yolk is followed by a lengthening in the same axis, and finally it assumes the constricted form. The first constriction may, however, disappear before it has accomplished the separation of the halves, to reappear only when two more nuclei have been formed and a second constriction has made its appearance, in a plane perpendicular to that of the first. Selenka considers this, as well as the usual method, normal, and is inclined to seek an explanation of the former in the hastened division of the primary cleavage nucleus.

Auerbach ('77) has given, in a paper which I have not been able to secure,* an explanation of the striation of the spindle. It does not necessarily originate in the same manner in all cases. If the granules imbed-

* This account is taken from Hofmann u. Schwalbe's Jahresbericht, Bd. VI., Anat. Abth., p. 25.

ded in the viscid substance of the nucleus are themselves viscid, they will be drawn out into filaments with thickenings at one end or in the middle according as the traction is from one or both sides. Thus it happens in the division of the sperm mother-cells of Strongylus auricularis that the nucleoli, arranged in rows, are converted by this stretching into meridional fibres; but if the numerous spherules are *movably* imbedded in the viscid matrix, then an elongation of the latter will cause the former to arrange themselves in parallel rows, which will give origin to a striate *appearance*.

Grobben (**'78**, p. 38, Taf. III. Fig. 17) has often seen the spindle-shaped nucleus with fibres and prominent equatorial thickenings in the large testis cells of Astacus.

In his paper on the organization and development of the Oxyuridæ, Galeb (**'78**[a]) has given an account of the nucleus during segmentation. In Oxyuris blatticola (p. 365) one finds eggs in the following conditions: non-segmented eggs with a germinative vesicle [primary segmentation nucleus?] near one of the poles (Pl. XXII. Fig. 1); others with the germinative vesicle elongated into the shape of a biscuit and in process of division (Pl. XXII. Fig. 2). Subsequently the germinative vesicle divides, and one then sees two vaguely rounded clear spots in the vitellus. The figure cited (Pl. XXII. Fig. 3) represents each of the spots as embracing a single nucleolus. The segmentation follows rapidly, and the halves of the "germinative vesicle" separate, the larger portion migrating to the opposite pole of the egg. The inequality in the size of the first two segmentation spheres may be attributed (p. 366) to this inequality in the size of the segments of the germinative vesicle; each portion of the latter probably exercising an attractive force upon the vitelline granules proportional to its volume. Although the author has seen the stellate figures in the eggs of other animals, he has never succeeded in doing so here, and concludes that they are concealed by transparent fatty vesicles, unless, however, their production is caused by the employment of reagents. In fact something of this radial structure was seen after using reagents in the case of O. Künckeli. Of a spindle metamorphosis of the nucleus, the author may be said to have seen nothing.

In the last chapter of his book, "Ueber das Ei," etc., Brandt (**'78**) argues further to show that the supposed disappearance of the germinative vesicle (and of the nucleus during segmentation) is due to the active amœboid changes of its form. "Die Theilung des Keimbläschens, sowie die der Furchungskerne, vollzieht sich unter wech-

selnden amœboiden Gestaltabweichungen (Strahlensonnen von Pseudopodien)." (p. 177.)

The inaccuracy of his conceptions of the nature of the "Strahlensonnen" is too apparent to need a special refutation. To consider these astral figures, which belong primarily to the protoplasm of the yolk, as pseudopodia of the *nucleus*, is to ignore the evidence of most careful observers; to claim that the nucleus is capable of automatic change of form, is quite another thing, to which I cannot object. How far Brandt is misled by the observations of others appears (p. 178) from the view held by him that the *membrane* of the germinative vesicle becomes the spindle of Bütschli, while its active substance escapes from the membranous enclosure at two opposite points. (!) The unreliableness of Brandt's conclusion, that the nuclear *reticulum* is only a pseudopodal extension of the nucleolus, and that the granules occurring in it are only local thickenings of the pseudopodia, has, I believe, been shown by the recent exquisite researches of Flemming and others.

Repiachoff (**'78**) gives rather unsatisfactory figures of the condition of the nucleus during cell division. Before each segmentation it becomes homogeneous, and the beginning of its division antedates that of the cell. In Figs. 10 – 12 the author reproduces the stages of nuclear division, showing the "dumb-bell" with a slender handle. More or less prominent asters, centring in the homogeneously stained heads of the dumb-bell, are figured. The heads are designated as "Theilstücke des Furchungskernes." Neither spindle fibres nor thickenings seem to have been observed, nor yet the incipient stages of nuclear formation, if, as is reasonable to suppose, this takes place in the manner now known to prevail with the eggs of most animals.

The fourth chapter of the summary on fecundation, segmentation, etc., given by Von Jhering (**'78**,, pp. 143 – 156), is devoted to the phenomena of segmentation and cell-division. It does not lie within the aim of his paper to contribute new material to the discussion.

Besides the method of cell increase which Auerbach denominates *palingenetic*, and which may safely be said to be the most wide spread of all forms, Von Jhering recognizes as different from it the free cell-formation of the botanists, and the equivalent methods in the segmentation of insect eggs and in the division of epithelial cells. As claimed by Strasburger, this latter method may be related to the former, in the sense that it is produced by a shortening of the process of development. A simple genuine division (i. e. without metamorphosis of the nucleus), from which budding is in no way fundamentally different, cannot be

denied, and appears to be realized especially in the case of unicellular Protozoa. Finally, the *endogenous* method of cell increase, though much less general than was formerly maintained, is not for that reason to be totally denied. It occurs more especially in the Protozoa and the Mesozoa (Dicyemidæ). Common to all forms of increase, save free cell-formation, is a continuity in the successive generations of nuclei. As Von Jhering's review of the *palingenetic* method of cell increase is simply a fair presentation of what appeared the more salient and unmooted features of the process, with possibly a slight tendency to magnify the observations of Selenka, I shall spare myself the trouble of reviewing his statements in detail. In so far as his paper is a reflection of the ideas prevailing at that time, it warrants me in calling attention to some points at variance with observations on Limax. Von Jhering appears to maintain a direct genetic connection between the radial figure of the spermatic nucleus and one of the two stars which constitute the amphiaster of the first segmentation sphere.* Although assenting that the asters are not always of the same age, I cannot agree, for reasons elsewhere expressed, that there is any basis for assuming the implied connection. The idea that the systems of yolk rays arise about the ends of the spindle, rather than that the spindle is a differentiation which may make its appearance somewhat later than the asters, is a one-sided view of the possible order of events. It cannot be doubted that the spindle fibres may far exceed the numbers (12 – 24) given by Von Jhering. It is perhaps premature to say that "the Zellplatte in the case of animal cells is of only theoretical interest"; at least, traces of the structure in question are so persistent in Limax as to compel the belief that not all of the substance of the interzonal filaments finds its way into the new nuclei.

In his "Zoologische Studien," SELENKA ('**78**, pp. 11 – 14) extends and somewhat modifies his preliminary communication. The incisions at the pole of the segmentation nucleus, at first thought to initiate its fibrous differentiation, are only phenomena of contraction in its welded, but not yet intimately fused parts [pronuclei, Van Beneden], and they

* "Den auf den Eikern zuwandernden Spermakern hatten wir als das Centrum für eine weitgehende Dotterstrahlung kennen gelernt, während dem Eikerne, nachdem er einmal seinen Platz im Centrum des Eies eingenommen, eine solche nicht mehr zukam. An dem Furchungskerne, kurz nach seiner Entstehung, gewahrt man daher nur ein Centrum der radiären Dotterstrahlung. Sobald nun der Furchungskern die Spindelform angenommen, erscheint noch eine zweite Sonnenfigur im Dotter, indem jeder Pol der Spindel zum Mittelpunkte eines Strahlensystemes wird." (p. 150.)

disappear as soon as the nucleus has taken on the genuine spindle shape.

Selenka believes he can add to the results reached by Auerbach, Strasburger, and Bütschli. Soon after the segmentation nucleus has taken an elliptical form, its substance is separated into a protoplasmic nuclear spindle, and a nuclear fluid surrounding it. The latter is so difficult to distinguish on account of the "clear area" which surrounds the nucleus, that it is easily overlooked, and hence arises the erroneous idea that the spindle represents the whole of the contents of the nucleus shrivelled. The nuclear fluid increases rapidly in amount, assumes a spherical form, and finally ruptures the nuclear membrane (already figured by Bütschli), and thus becomes mingled with the surrounding yolk.

During the fission of the nuclear plate a small lustrous corpuscle lies at the tip of each end of the spindle. Whence it comes, Selenka cannot say, nor can he add anything concerning the origin of the spindle fibres or their thickenings; but the segmentation nuclei of the *second* generation arise substantially from a fusion of the elements of the nuclear plate, without the participation of any other formed structure.

From this it may be inferred that the corpuscles at the tip of the spindle do *not* enter (at least without first suffering dissolution) into the composition of the new nuclei. I fail to see, however, that the observations, so far as the figures allow one to judge (Taf. III. Figs. 18–20), are competent to prove this exclusion. If the exclusion of these corpuscles were established, and if they are identical with the corpuscles occupying the *centre of the polar areas* in Limax, (an identity which I do not feel to be fully justified in view of their prominently eccentric position in the terminal suns,) then I should find in Selenka's statement a confirmation of the opinion I have elsewhere ventured, viz. that the areal corpuscles do not directly share in the formation of the new nuclei.

The nuclear fibres (14–24 in number) are at first separated from each other by equal intervals; but when the fibre thickenings have advanced to near the ends of the spindle, and have fused in pairs, these secondary thickenings take the form of a circle [ring?] and further unite, as has been stated in the review of the preliminary paper. Instead of *Vorkerne* used in the earlier communication, Selenka suggests the use of *Nucleoplasts* for the "Kernfaserelemente," since the former name (pronuclei) is otherwise employed. When the "nucleoplasts" are six in number at each pole, they have the form of conical rods with the tips directed outward, their blunt ends united to the interzonal filaments.

After the formation of the segmentation nuclei of the second genera-

tion, the radial figures disappear very rapidly, but the clear area in which each nucleus is imbedded persists.

The primary cleavage nucleus in Clepsine is found to lie, according to WHITMAN ('**78**[a]),* "a little eccentrically toward the oral pole. The nucleoplasm is more strongly colored in the centre around the pronucleolar bodies than at the edges." There are usually three of these "pronucleolar" bodies, which are "sharply outlined, but only slightly stained with carmine." "The longer axis of the nucleus in this stage is in every instance at right angles to the axis of the egg, whereas, at the moment of union of the pronuclei, the longer axis was found parallel to that of the egg, and a little later inclined about 45°." The "pronucleoli" are several times larger than when the pronuclei meet. Between the nucleus and the oral "polar ring" a line, more highly colored than the rest of the yolk, is sometimes seen. This, from its position and direction, Whitman interprets as the path taken by the female pronucleus toward the male pronucleus. It occurs to me that it may be the same structure which Fol has seen in the Pteropod egg.

The nucleus elongates and then passes from a spindle- to the biscuit-form. The "nucleoli," having dissolved, are no longer visible, but there stretch through the centre of the biscuit-shaped figure "fine granular lines, which together form a sort of spindle, the poles of which appear to be near the centres of the polar areas." These interstellate lines are more strongly expressed than the radial lines of the two polar areas.†

At this time, says the author, the substance of the "polar rings" — for an account of which the reader must be referred to the interesting description given in the original paper — begins to plunge into the yolk, and possibly contributes some elements to the nucleus which may stimulate the molecular changes which result in the formation of the cleavage amphiaster.

Subsequently, there arises in each pole of the amphiaster "a *central area* ‡ which colors less with carmine than the surrounding nucleoplasm, and in the edge of which the converging rays end." These central areas undergo a modification from the round to the biconvex, and finally to

* Compare also Whitman '**78**.

† The eggs of Clepsine appear to be very unfavorable for the study of the nuclear plate, for Whitman was unable to find any trace of it during the first segmentation.

‡ This central area in a more advanced stage the author identifies with the cell *nucleus*. His "nucleoli," therefore, correspond with what many observers consider the still unfused elements of a nucleus, and his "nucleus" with what I have called "area." A discussion of his views on this point will be found under Fecundation (pp. 504 *et seq.*).

the meniscus form, during which changes they move in opposite directions, as if repelling each other. The spindle fibres gradually disappear.

At the completion of cleavage a cluster of four to six refractive "nucleoli" is formed in each of the areas, and the latter at that time begin to approach, again passing through the same series of forms, but in the reverse order.

Although not directly stated, it is to be inferred that these nucleoli do not fuse until the formation of the second segmentation spindle.

In the fourth instalment of his "Beiträge," etc., O. Hertwig (**'78**[a]) has devoted some attention to the phenomena accompanying the first segmentation.

Living eggs of Mitrocoma seem to become enuclear by the sudden disappearance of the mutually flattened pronuclei, but the use of acetic acid establishes the presence of a fibrous spindle which is eccentric in position and is accompanied by radial arrangements of the yolk at its tips. The division begins, about two hours after fertilization, with a furrow which makes its appearance at a point of the surface directly over the spindle, and is accompanied by the formation of secondary smaller folds in the cortical substance at right angles to the segmentation furrow. A number of small vacuoles — the metamorphosed halves of the nuclear spindle — make their appearance in each segment when the furrow has advanced as far as the middle of the yolk. The portions of the yolk last separated are those lying diametrically opposite the side occupied by the spindle (p. 183).

The observations on Tiedemannia (p. 205) are more interesting since the origin of the amphiaster is more successfully traced. The pronuclei remain a long time close to the surface in an unaltered condition. At length their nucleoli become disintegrated into clusters of smaller granules, which collect themselves on either side of the conjugating surfaces of the pronuclei (Taf. XI. Fig. 5). Then two systems of faint rays make their appearance at opposite edges of the surface of contact. Suddenly the contours of the nuclei become indistinct, and both the vacuolar spaces suddenly disappear, probably through a mingling of the surrounding protoplasm with the nuclear fluid. In the homogeneous protoplasm one may still barely recognize the two systems of rays at some distance from each other. The division follows much as in the medusa alluded to above.

Although the contour is figured somewhat less boldly where the two nuclei are in apposition, it is not stated that the nuclei actually unite

before the disappearance of the whole outline, and I am inclined to think this case is closely related with the condition found in corresponding stages in Limax, where the evidence strongly favors the view that there is not an actual union of the pronuclei preceding the appearance of the asters. It is noticeable that Hertwig makes no mention of the spindle in this case, doubtless from its tardy appearance, as in Limax. He does not seem to have discovered the asters at any point other than those which lie in the plane of nuclear contact, nor that they may come into view successively. In both particulars the eggs of Limax have proved more favorable in determining, if not the prevailing, yet at least the possible, order of the events of nuclear metamorphosis.

In each of the conjugated nuclei arises in Pterotrachea a single, or in Phyllirhoe numerous nucleoli, which are, however, of only temporary duration. After their disappearance two radial figures arise, as in Tiedemannia, but in the present case the partition between the two nuclei disappears, so that an actual fusion takes place. In the nuclear space thus made common, fine fibres stretch between the two suns. Later, the contents of this nuclear vacuole, which in Hertwig's opinion contains in addition to the spindle nothing but nuclear sap, become mingled with the surrounding protoplasm, whereby the comparatively small spindle comes to lie free in the yolk (pp. 208, 209).

In Pterotrachea, as well as in Mytilus, the activity of the vegetative pole of the egg just before or during the first segmentation results in more or less conspicuous protoplasmic elevations of the yolk.

Very recently Bobretzky (**'78**[a]) has published observations on early stages in the development of insects. He maintains that the blastoderm cells in certain Lepidoptera are formed within the yolk by a process of cell division, and migrate as amœboid cells — hitherto mistaken for nuclei — to the surface, where they successively make their appearance to form the blastoderm.

Lang's (**'78**) studies on the development of Balanus contain indications that in the segmentation the nucleus undergoes a spindle metamorphosis, but give no data concerning the maturation or fecundation, save that a segregation of the ectodermal protoplasm at one pole precedes the "unequal segmentation."

Hatschek (**'78**) has given little attention in his excellent paper on the development of Annelids to the details of cell division. He says, however, (p. 17,) that one very often finds in the primitive cells of the mesoderm indications of division, — spindle-shaped nuclei, and granular rays in the protoplasm.

β. *Tissues.* — Observations upon metamorphosed nuclei of *tissue* cells, certainly referable to a *process of division*, are mostly of quite recent date, and the papers which treat of them are largely the result of the stimulus afforded by discoveries in connection with segmentation.

The papers of Bütschli and Strasburger in 1875 served to recall the attention of MAYZEL (**'75**)* to certain appearances of nuclei which he had often observed in his studies on the regeneration of epithelium, and which up to this time had remained problematic to him. In essential agreement with the observers alluded to, he at once came to the conclusion that the existence of numerous coarse granules and fibrous structures in the nuclei was connected with the process of nuclear division.

The cornea of the rabbit and the cat, but more particularly the cornea and other epidermal structures of the frog, were the objects studied. Mayzel was unable to confirm by studies on fresh specimens the results attained in the use of different reagents. Although occasionally observed in regions of normally preserved epithelium, the appearances were most frequently met with in the tracts where regeneration had followed an artificial removal of the epithelium, and then not at the edge, but in the midst, of the regenerated portion, and in the deep rather than in the superficial layers. He distinguished three principal forms of the nucleus, without, however, being able to affirm positively that they follow one another in the order in which they are described : —

(1.) Large oval nuclei of twice the diameter possessed by their neighbors, either coarsely granular at the periphery only, — thus disclosing the nucleoli, — or throughout ; then such as have their granules elongated into threads, and knotted together ; and finally those with similar filaments, alike in thickness but of various lengths, arranged radially about a central point.

(2.) Large, elongated, spindle-shaped nuclei, with a thick transverse disk which appeared either more nearly homogeneous, or else as if composed of coarse, refringent granules of unequal size. Occasionally the disk appeared double. The remnant of the indefinitely outlined nucleus was delicately fibrous in the direction of the long axis, the fibres so converging at the ends of the nucleus as to give it the appearance of two fibrous cones placed base to base.

* As I learn from Flemming (**'75**, p. 186) and Strasburger (**'76**, p. 230), KLEBS (**'74**) had already noticed early in 1874, in studying the regeneration of epithelium, a radiate arrangement of the protoplasm which he connected, however, with the genesis of new, rather than with the division of previously existing, nuclei.

(3.) Nuclei of like size, but more elongated, the two ends consisting of two saucer-shaped structures with their cavities facing, and the intervening portion occupied by numerous filaments differing in thickness stretched between these two structures. The "saucers" appeared at times as though composed of radiating filaments, at others as though made up of a nearly homogeneous, lustrous substance. The equatorial disk is no longer visible. The distance between the saucers is now more, now less. The connecting fibres rupture successively. The "saucers" become flattened into disks, and appear either as a mosaic of rods or else homogeneous. The disks become thicker and rounded, and acquire vacuolar cavities in which nucleoli appear. They now nearly resemble the neighboring nuclei.

Accompanying changes in the shape of the cell and its constriction ultimately end in cell-division, the nuclei, at first close to each other, sometimes appearing to be still joined by fine filaments. They subsequently move apart, and the cells, like those which surround them, become polygonal.

Mayzel ventures the statement that, at the free edge of the regenerating patch of epithelium, the nuclei are without doubt formed [not by division but] by differentiation out of the protoplasm.*

The observations of Ed. van Beneden (**'75**) on ectoderm cells of the rabbit embryo are to be found at pp. 302, 303.

Semper (**'75**[c], pp. 361, 362, Taf. XIX. Fig. 29, x), among the "Ureier" of Acanthus, found some whose nuclei were smaller than usual and appeared to be composed of small granules often radially arranged about a centre. Such nuclei are more deeply stained in hæmatoxylin than the ordinary nuclei. Semper is inclined to connect them with the process of cell division, especially in view of their close similarity to the phenomena accompanying segmentation, as shown by Bütschli, Auerbach, and Flemming.

Ewetsky (**'75**) seems, according to Strasburger (**'76**, p. 228), to have figured something of the phenomena of nuclear division. I have not been able to consult his paper.

Besides his study of the blastoderm cells of insects, the results of which have already been given (p. 320), Bütschli (**'76**, pp. 249–262) extended his investigations to an examination of cell and nuclear division in the germ cells of the spermatozoa of Blatta, and in the blood-corpuscles of the fowl, the frog, and Triton. The more important

* "Dass die Kerne ohne Zweifel durch Differenzirung aus dem Protoplasma sich frei bilden."

results, as far as regards Blatta, have been given in connection with the review of his preliminary article (p. 289), and I will here call attention to only one or two points dwelt upon in this ultimate paper.

The nuclei of the "great germ cells" exhibit, after the employment of acetic acid, numerous dark granulations which are united to each other by fibres, — strung on the latter, as it were. These fibres may constitute an irregular net work, running through the whole nucleus, or they may take a quite regular, bush-like form. In the latter case they arise from a point of the nuclear membrane about which granules of the cell protoplasm are grouped. In clusters of neighboring nuclei these points are turned toward each other, a fact which leads to the conviction that a process of nuclear multiplication takes place with these "great" cells. This opinion is strengthened by finding two such nuclei still connected by fibres. After these "great" cells had become isolated from each other, the nuclear metamorphosis was followed in the *living* cells, as far, at least, as to establish a fibrous nature for the elongated oval nucleus. Occasionally an equatorial thickening was also visible.

There is little to be seen in his figures of a central area, though the radial structure is well shown in some cases. (See Taf. V. Figs. 14, 17, *op. cit.*)

The daughter nuclei arise by a differentiation out of the dark homogeneous nuclear plates (Kernplattenkörper). This differentiation is to be considered as most probably brought about by the accumulation of fluid between an external layer (nuclear envelope) of this dark, homogeneous body and its central portion (nucleolus). This originally single nucleolus *suffers a disintegration* into a number of parts, each still connected with interzonal filaments. Nothing like a cell plate is to be seen.

The very interesting statement is made that sometimes masses of protoplasm containing *two* nuclei undergo division. This was observed to take place in one or the other of two ways. In both methods the two nuclei divide at the same time and give rise to two spindles having parallel axes; in one case the protoplasm is grouped about the two daughter nuclei, which occupy corresponding ends of their respective spindles, as if about a common centre; in this case two binuclear cells result; in the other case the components of *one* set of daughter nuclei may separate from each other and become the centres of *two* smaller cells. In the latter case the result is one binuclear and two uninuclear cells.

Bütschli draws the conclusion that a common cause, resident only in the surrounding protoplasm, affects the division in these cases.

The blood-corpuscles of the embryo fowl, on the fourth or fifth day of incubation, when treated with reagents, furnish satisfactory proof that a nuclear metamorphosis accompanies cell division. The so-called equatorial plate may in this case be composed of distinct rods, or may be an actually continuous plate as in many plants. The spindle as a whole is so large as to lead to the belief in an *increase* in the volume of the original nucleus. Faint traces of a second equatorial thickening are found and interpreted as a cell plate.

Regarding the structure of the nuclei of blood-corpuscles Bütschli is (p. 260) at variance with Auerbach (**'74**, pp. 61 – 70, 98, 103, 114) inasmuch as he finds that the nuclear substance exists in the form of irregular fibres, with nodose thickenings in many places, which traverse the nucleus and are united with its envelope, rather than as discrete nucleoli. The division of the white blood-corpuscles in Rana and Triton is accompanied by changes of the nucleus which present only a remote resemblance to the typical metamorphosis of this structure. There is simply an elongation of the nucleus, and a gradual swelling of its ends; while the middle, connecting portion becomes attenuated to a fine thread, which probably ruptures and becomes incorporated in the two daughter nuclei.

Strasburger's (**'76**, pp. 208–211) observations on nuclear division in animal tissues were limited to studies on fibrous cartilage from the ear of the calf. Notwithstanding the unfavorable nature of the object, — preparations from which leave much room for the play of the imagination, — Strasburger believes the division takes place by the lengthening of the nucleus, and the formation of an equatorial plate whose halves separate and leave stretched between them "nuclear filaments," before there is any sign of a division of the protoplasm. In the equator of the nuclear [interzonal] filaments, and in the surrounding substance as far as the wall of the cell, there is then seen a dividing layer, — the beginning of the cell plate. This partition is not formed progressively from the circumference inward, but is produced simultaneously throughout its whole extent, and subsequently splits, as in plant-cells, into three layers, of which the central forms the fibrous intercellular substance of the cartilage.

Studies by Eberth (**'76**), immediately induced by Strasburger's work, and carried on without a knowledge of what Mayzel had done, corroborate most of the conclusions of the latter author. On the cornea of the rabbit and the frog Eberth has also shown that after artificial removal or destruction of the epithelium the ensuing regeneration affords oppor

tunity to study nuclear division, and usually at some distance from the injured spot. Also on preparations of the normal cornea made with gold chloride, certain nuclei appear larger than the others, and are composed of clusters of lustrous corpuscles, somewhat smaller than the smallest nucleoli. In addition there are found simply curved and S-shaped lustrous rods of the same diameter as the granules, with knoblike ends. These are often radially arranged.

In the rabbit's cornea undergoing regeneration occur similar phenomena. When the granules are not too close together one sees between them the larger nucleoli. Moreover, only a part of these granules are free like the nucleoli; others appear as small thickenings in the netlike system of filaments which form the nuclear stroma. In other cells the nuclear membrane and the nucleoli are no longer to be found, and in place of a granular nuclear substance there is an irregular, lustrous, starlike structure, half as large as the nucleus previously was. The rays of this star are either plump, or, if slender, have terminal swellings, and may be elongated and spindle-shaped. A clear area, surrounding this figure, more or less sharply defined from the surrounding protoplasm, is the remnant of the previous nucleus. The fibrous mass becomes shortened and thickened, and thus assumes the shape of a double convex lens, or a sphere, of meridionally arranged rods and granules. An equatorial fission separates this structure into *hollow* hemispheres, — each a sort of fibrous basket. The granules have now become less abundant, having probably been converted by fusion and elongation into filaments; at least the latter are more numerous than previously. The parting filaments are often swollen, at other times they end in attenuated extremities. They vary in length, and may anastomose with each other. The separation is not effected at the same instant in all the fibres, a part retaining their connection for some time. The basket structures separate, and after the parting of the last delicate traces of the equatorial ends of the fibres their peripheral ends terminate in a cluster of lustrous granules, or a crescent-shaped body, and are no longer distinguishable from each other. The crescent-shaped body grows at the expense of the fibres, and there results in place of the fibrous basket an oval, jagged body — the new nucleus — surrounded with a clear area. This new nucleus is a sort of shallow cup of homogeneous substance. The clear area is the result of the constriction and division of the area which previously surrounded the two fibrous baskets. The cell undergoes division during the conversion of the basket into a homogeneous, jagged body. The latter subsequently changes into a larger vesicular nucleus.

In spindle-shaped cells of the cornea (rabbit) a fine line which traverses transversely the space between the two incipient nuclei (*op. cit.*, Taf. XX. Fig. 1. *d*) is thought to be an indication of the coming cell division. It may, then, I would add, correspond to Strasburger's cell-plate. Similar changes accompany the division of other cells, and are very conspicuous in the frog's cornea. In the Descemet's cells of the latter the fibrous mass is sometimes divided at once so as to give rise to *four* new nuclei, perhaps in some cases to six or seven.

The conclusions drawn by Eberth are, to epitomize: — Many cells do not divide simply, but first undergo a metamorphosis which begins with an enlarging of the cell and its nucleus. The latter, by absorption of fluid from the cell protoplasm and by differentiation into clear fluid (Saft), lustrous granules and filaments, becomes lighter. The granules are *not* derived from the nucleoli, however much they resemble them, for the two coexist. The nuclear membrane is dissolved, but nevertheless a mingling of the nuclear matter with the protoplasm does not take place. Since the granules and filaments often appear before the dissolution of the membrane, a complete karyolysis (Auerbach) does not take place. The fibres and granules form a jagged, or globular body, or a spindle figure, whereupon the fibres usually take a regular meridional course, but may remain quite irregular. In the latter case a more regular arrangement is only apparent after the beginning of the division, which takes place as above described. The fibrous mass, which has arisen in the mother nucleus, is the new nucleus, and gives rise by division to two (or sometimes four or more) daughter nuclei. The mother nucleus, in some cases at any rate, is still present in the later stages of metamorphosis, and is converted into the substance of the daughter nuclei.

The principal points of difference in the changes of corneal cells as compared with plant cells (Strasburger) are: — The differentiation does not (cornea) begin with longitudinal streakings, but at once with the appearance of the equatorial granules and filaments, which furnish, if not always the whole, certainly a great part of the material for the new nuclei. In the young, cup-shaped, homogeneous nucleus reappears later a differentiation into clear fluid and anastomosing filaments, which latter then undergo a granular disintegration or are changed into a net of fine "Bälkchen" and thus form the stroma of a new nucleus. Thereby the nucleus appears granular. It then increases in size at the expense of the substance of the old nucleus, whose fluid is probably absorbed by the young reticular nucleus. In the corneal epithelium of

the frog, however, the greatest part of the substance of the old nucleus seems to be employed in forming the filaments, and the little that is left appears to be mingled with the surrounding protoplasm; for, *before* the division of the new nucleus into the daughter nuclei, the clear remnant of the old nucleus has disappeared.

Balbiani ('76) has studied the nuclear changes during cell division in the epithelial cells of the ovary of an orthopterous larva, — Stenobothrus. The nuclei of these cells do not embrace nucleoli, in the sense generally given to that word; but in the fresh condition the whole interior of the nucleus appears filled with little faint "hatchings" (hachures) either parallel or irregularly arranged, — an appearance such as would be produced by bacteria. The use of acetic acid shows this appearance to be due to straight, rod-like corpuscles which the acid makes refringent. Under a high magnifying power each rod seems formed of small globules united into rows. As the cells multiply the corpuscles become successively smaller, so that the nuclei in the walls of an ovarian chamber enclosing a nearly ripened egg contain only a mass of fine granulations. With approaching division the rodlike corpuscles of the nucleus become larger and less numerous; instead of being rectilinear they present flexures, curved in various directions, or even short ramifications. These large rods (bâtonnets) appear to Balbiani to arise by the agglutination and reciprocal coalescence of the primitive nuclear corpuscles. The cell and nucleus become ellipsoidal; the rods form a loose bundle parallel to the long axis of the nucleus. Then they appear as homogeneous, cylindrical, or fusiform rods (baguettes) extending the whole length of the nucleus. Soon each is constricted in the middle, and is then divided into halves so that two smaller bundles result from the primitive one. These move apart along a rectilinear course, but there is not a complete separation because a delicate filament continues to unite the halves of each rod, and taken together these filaments give a distinctly striate look to the modified nucleus. The cell becomes narrow and elongated; the peripheral contour of the nucleus completely disappears, so that the body formed by the rods and filaments appears plunged directly in the protoplasm of the cell and surrounded at a little distance by the contour line of the latter.

During the separation of the two bundles their component rods become approximated and [their substance becomes] mingled at the distal ends, but at their proximal ends they separate from each other, so that each bundle takes the form of a cone, the two bases facing each other. The summit of the cone becomes rounded into a sort of cupola with a

dentate border caused by the unfused portions of the rods. By the constriction and division of the cell the filaments are cut in the equatorial plane, and withdraw into the mass formed by the rods, now completely fused. This mass is at first homogeneous, then small vacuoles appear, a membrane becomes perceptible at its periphery, and the contents are resolved into rodlike corpuscles like those which the nucleus contained before division.

The nuclear equatorial granules were only rarely seen, and then they took the place of the rods, each sending a filament to each pole of the nucleus. They are only local accumulations of the substance of the rods, which is withdrawn from the poles to be concentrated in the equatorial region, — simple varicosities of the filaments. Balbiani was unable to observe the radial phenomena of the cell protoplasm, on account, as he thinks, of its great homogeneity.

Mayzel (**'76**[b]) * distinguishes two forms of nuclear division in epithelial and other tissues of numerous animals which he has studied. In one form — constantly exhibited by the endothelium of the cornea (frog) — there is a spindle-shaped structure, which is divided, he says, into two cones by a median transverse nuclear disk; from this nuclear plate numerous fibres extend to the apices of the cones, quite the same as seen by Strasburger and Bütschli in Blatta, etc.

The rods and granules, however, which compose the nuclear disk do not appear in Mayzel's preparations † as thickenings of the nuclear fibres, but form, in some cases at least, a kind of ring *surrounding* the spindle, the latter being composed of fibres alone. One may therefore assume, I think, that the elements which form the nuclear disk arise from compacted nuclear substance which is *independent* of the nuclear fibres.

In the germ cells of spermatozoa in Blatta, the nuclear plate is composed of granular thickenings of the nuclear fibres, just as described by Strasburger and Bütschli. A comparison of fresh specimens with those that have been treated with reagents (chromic acid 0.01%) shows that the poles of the spindles, which are rounded in the fresh condition, become more pointed, the nuclear fibres thicker, the whole nucleus narrower, and that a clear zone is formed about the nucleus. Because this space is artificially produced it cannot be considered a remnant of the old mother nucleus (Eberth), in which the fibrous mass is differentiated as a new nucleus. While the elements of the nuclear plates fuse at the

* See also Mayzel **'76, '76**[a-d].

† Compare Strasburger (**'77**, Taf. XXXIII. Figs. 56 – 61), where figures of Mayzel's preparations are given.

poles of the spindle into two new nuclei, there is formed about each of the latter a deeply staining homogeneous zone. It appears to be produced from the nuclear fluid, which is pressed out toward the poles, and then consumed in the growth of the new nuclei. In no stage of the division did Mayzel observe a radial arrangement of the protoplasmic granules, such as is to be seen in the eggs of Ascaris.

The second form of nuclear division was seen in the endothelium from the cornea of the frog, and the epithelium from that of the rabbit. This appears like an hour-glass composed of fibres. The body of the cell may either remain unaltered or may be constricted, in which latter case the constriction closely invests the narrow part of the nucleus. The latter corresponds to the "Kernstränge" of Bütschli as seen on germ cells of spermatozoa. In the corneal *epithelium* of the rabbit, bird, etc. there is formed, independently of the gradually disappearing nuclear fibres, a *new* equatorial partition composed of granules. In the corneal *endothelium* of the frog, on the other hand, there appears in the equator of the cell between the nuclear fibres a row of small interstices or vacuoles, which appear to be filled with a cementing substance. It is from the union of the contents of these vacuoles that the partition arises. A simultaneous division of a nucleus into seven, or even into four parts (Eberth), was never observed. All the important phenomena of the process of nuclear division as given above were also seen in fresh preparations of the frog's cornea examined in aqueous humor.

The observations of Fœttinger (**'76**, p. 607) on the division of nuclei in epithelial cells of Petromyzon give no evidence of the existence of a fibrous nuclear spindle.

Bütschli's (**'77**[a], pp. 212–214) studies on the division of cartilage cells were successful only in making it probable that the nucleus of such cells undergoes a fibrous differentiation preliminary to constriction, and were inadequate to establish any close relation between the nuclear division here and in the typical cases of spindle metamorphosis. He therefore finds himself forced to the conclusion that a direct comparison of the two methods is not for the present attainable, although the similarity of the process to that which obtains for the secondary nucleus of Infusoria makes it reasonable to believe that, in the present case, one has to do with a modification of the primitive method of nuclear division.

In a subsequent communication Mayzel (**'77**[a]) extends his observations to the eggs and young stages of Triton and Perca. In segmentation spheres of the fish egg from 75 μ to 25 μ diameter, the figures of nuclear division differ from those previously reported for the endothelium

of the frog's cornea only by the presence of a distinct radial arrangement of the granules in the cell protoplasm. The processes in the epithelium of the tail of the fish embryo were essentially as in the epithelium of the frog's cornea. The Kernplatte were distinct, and appeared after division like two combs with the teeth turned toward each other.

In the case of Triton the division is similar. The granules and fibres of the modified nucleus are remarkably thick.* After division the nuclei take the form of fibrous baskets destitute of bottoms, and when stained in picrocarmine appear like "gayly colored flowers." The nuclear plate is wanting. The staining of the fibres in picrocarmine and their failure to do so in osmic acid are evidences to the author that they are simply modified nuclear substance. This reaction, coupled with the contrary deportment of the remnants of "Dotterplättchen" to be found in the larva of Triton, is sufficient proof of the inaccuracy of Török's conclusion, that similar filamentous structures in Siredon arise by a metamorphosis of the Dotterplättchen. Moreover, the nuclear division is of the same nature in the fully developed Triton, where *no* Dotterplättchen are present.

Further, the direct observation of division in living cells and nuclei (epithelium of Triton larvæ) is possible. Two cells were seen to divide in the course of ten minutes, in one case by an advancing constriction, in the other by means of the formation of a row of small vacuoles in the equator of the cell. The nuclei were already divided, and appeared in the form of a basket composed of dull lustrous rods conically arranged. The rods become shorter, first growing more slender at the equatorial and thicker at the peripheral end, and finally fuse into an irregular knobbed mass. These changes of nuclear form cannot be called amœboid.†

Strasburger (**'77**, pp. 519–521, Taf. XXXIII. Figs. 56–71) has introduced into this paper several figures of Mayzel's preparations illustrating division in the tissue cells of animals. The elements of the nuclear plate (Figs. 56–58) from endothelium of the frog's cornea exhibit the same arrangement (outside the spindle fibres) which Strasburger pointed out as sometimes existing in Nothoscordum. A well-marked

* Compare Strasburger (**'77**, Taf. XXXIII. Figs. 64–71) where Mayzel's preparations are figured.

† See also Mayzel **'77** and a review (**'77**[b]) by the author himself in "Hofmann u. Schwalbe's Jahresbericht," etc.

P. S. — Another more extended paper by Mayzel (**'78**) is inaccessible to me, and the short notice (Mayzel, **'78**[a]) given of it in the Jahresbericht of Hofmann and Schwalbe is too brief to be of any value. A review of the whole is promised when the second part has been published.

area surrounding the spindle figure is interpreted (see also Eberth) as marking the limits of the unmetamorphosed nucleus, and is believed to be filled with nuclear fluid. This area is less common in plant than in animal cells.

The position of the nuclear [interzonal] filaments at an advanced stage of cell division (that is, whether they converge toward the equator or not) indicates the method by which such division is accomplished, — whether by a constriction, or by the formation of a cell plate simultaneously through the whole equator of the cell, or by a combination of both methods. In the last case a constriction advances from the periphery, but proceeds only till it reaches the circumference of the spindle, when its work is supplemented by the splitting of an already formed cell plate. The two first-mentioned methods are respectively exemplified, in Mayzel's preparations, by endothelium from the frog's cornea and epithelium from that of the sparrow; the third method, by Dicyema germs as described by Ed. van Beneden.

Peremeschko (**'78**, **'78**[a], **'78**[b]) gives in preliminary communications the results of his studies on cell division in the case of Triton cristatus in (1.) the epithelium of the body; (2.) star-shaped connective-tissue cells; (3.) white blood-corpuscles; and (4.) endothelium of blood capillaries.

The process of division is in all cases the same. In the centre of the cell appear first small, and then large granules. These change into thicker or more slender threads, distributed at first without order. From the threads are produced structures which often have a quite regular form, — star-shaped, half star-shaped, knotted, etc. These forms are continually changing, and the fibres meantime are now pale, now sharply marked, now longer, now shorter, now finer, now thicker. They exhibit no locomotor motion. After these changes they assume a regular cask shape, and become thicker in the middle. (The thickenings do not all lie in one plane.) The fibres divide in these thickenings, and the cask thus separates into two similar portions, which at once move apart. Thus the two new nuclei are formed. The contour of the cell then becomes sharper, the protoplasm less transparent, — more compact, as it were, — and a furrow, corresponding in position to the space between the new nuclei, makes its appearance, at first on one side and subsequently on the other. The nuclei meanwhile continue for some time to change form, and at length the constituents of their polar ends melt together. They subsequently become pale, and finally disappear.

Flemming (**'78**) gives in the present paper an extract from a lecture,

which is a preliminary report on the results of studies upon the structure of the cell and upon the phenomena of cell division. He maintains in all essentials the views previously expressed (see p. 264) concerning the structure of the quiescent nucleus.

As this was not received till after his final paper (Flemming **'78**[b]) had been reviewed, the reader is referred to the account of the latter on page 355.

Schleicher (**'78**[a])* recognizes in cartilage cells capable of, but not undergoing division (Theilungsfähig), fibres, rods, and granules in the protoplasm outside the nucleus. The fibres are rectilinear or curved, often radially disposed, at other times concentrically arranged about the nucleus, etc. The rods are shorter than the fibres, but are also often radially disposed. The granules are most abundant near the nucleus. All of these elements exhibit lively amœboid motion, while the nucleus shows as yet no differentiation. This quiescent nucleus is either homogeneous with one or two clear nucleoli, or, if less homogeneous, it is doubtless owing to an approaching or just completed division. For Triton, however, the presence of coarse granules and rods in the nucleus is a *permanent* feature. But such structures do not necessarily involve the assumption of a *connected network*, against the existence of which, either in nucleus or protoplasm, the author urges the great activity of the structures in question. Their supposed existence and the union of intra- with extra-nuclear networks are referable to the employment of reagents. The histologist must confine (!) himself to the living cell in studying these phenomena. Since, in the author's opinion, all those structural peculiarities of the *nucleus* known as fibrous mass, granular mass, rodlike structures, stellar figures, glomeruli, etc. are only interchangeable appearances of the same thing, viz. "nuclear substance," he would designate the whole series of phenomena under the head of "Karyokinesis" (nuclear motion). In the formation of the karyokinetic figure participate granules and rods (when previously existing), nucleolus, *new* differential products, and the dismembered nuclear membrane. No change of dimension either in nucleus or cell heralds the approach of the division. The karyokinesis consists in a series of rearrangements of the nuclear substance without predetermined order, in which more or less regular figures are preceded and followed by such as are altogether irregular. These rearrangements are accompanied by the disintegration of rods into granules, and the reverse process. These changes — introductory to the real act of nuclear division — take place with varying

* See also Schleicher **'78**.

rapidity, but have not been known to consume more than two hours. One is not warranted in dividing the phenomena into a number of stages, for the character of regularity is wanting. Aside from these motions which affect the constituents of the nucleus, and are of primary significance, its whole mass in many cases undergoes a more or less irregular motion of translation through the protoplasm of the cell; and it may require from three to eight minutes for the nucleus to traverse the diameter of one of the larger ones. This motion ceases some time before the division of the karyokinetic mass. Another phenomenon, observed only in the frog, and of secondary importance, is the rotation of the nuclear mass through an arc of 90°.

During these changes in the nuclear substance the delicate rods and fibres of the cell protoplasm continue their motion and gradually tend toward the centre, where they are believed to undergo an assimilation with the nuclear substance. By this means they become more highly refringent, and therefore indistinguishable from the constituents of the nucleus.

The division of the nucleus is introduced by the sudden appearance of a parallel arrangement of the nuclear rods, which together form a somewhat elliptical cask-like figure. The rods at once divide and the halves move apart rapidly. The immediate destiny of the halves is the consolidation of their constituent half-rods, and the successive forms which may be assumed in the course of this process are quite as free from regularity as are the karyokinetic figures. Between the separating halves of the nucleus one may usually distinguish fine, clear internuclear fibres, which, although composed of fine granules, appear as continuous filaments. The author distinguishes from these other fibres or rods which stretch between the two halves, which have nothing to do with the internuclear fibres, and which soon disappear. He has given only isolated descriptions, which seem directly comparable with nuclear division as observed in segmentation spheres. The single observation of a spindle figure with equatorial accumulations of nuclear substance is interpreted as probably an accidental product. A genuine solar figure (which, however, is interrupted for several degrees of arc), whose fine rays stretch from the karyokinetic mass to the periphery of the cell, is distinguished by the author from isolated groups of stouter radial fibres: the latter are identical with the previously mentioned fibres of the cell protoplasm (*loc. cit.*, p. 277); the finer, on the contrary, are processes of the karyokinetic mass.

The consolidation of the constituents of the new nuclei advances from

the poles of the cask-shaped figure; this fusion results in the production of a *homogeneous* structure, which may have a somewhat irregular outline, — traces of its origin from distinct rods, — but which is only a transitional stage in the formation of the new nucleus. This homogeneous structure breaks up into elements which exhibit the same irregular karyokinetic phenomena as the nucleus *approaching* division. Ultimately a part of the stout fibres arrange themselves to form a nuclear membrane, which, however, does not enclose all the remaining nuclear elements, so that a part of the karyokinetic mass in each half is not employed in forming the new nuclei.

A summary of Schleicher's theoretical considerations is not easily brought within narrow limits. The reader must therefore be referred to the original for a complete exposition of the views entertained by the author.

In the metamorphosis of the old nucleus into a karyokinetic mass, the chemical outweigh the purely physical activities; new chemical products make their appearance; it is not a simple mechanical separation of the less from the more fluid constituents.

The membrane and the "nuclear stuff" (i. e. the *inner* nuclear substance) differ chemically, for the latter must suffer a chemical change before the two can unite. The growth of the nucleus during the karyokinetic period, by which a division is made possible, is at the expense of the enumerated protoplasmic structures; the latter, in turn, arise by a process of chemical differentiation, which is most active in the periphery of the cell. The growth of the nucleus is not brought about by a process of intussusception, but by the juxtaposition of visible granules. During the protracted karyokinetic period, unknown physical forces arise, which become recognizable at the moment of division in the sudden separation of the halves of the structure by *repulsion.* The assumption that the division takes place in obedience to two centres of *attraction* formed at the middle of each of the prospective daughter cells, is negatived by the fact that the position of such centres of attraction are conditioned by, and dependent upon, the distribution of the protoplasm, — the shape of the cell, in other words. It follows that in an elongate cell these centres would lie toward the poles, and the division would therefore have to take place in the direction of [perpendicular to?] the longest diameter; this, however, the author's observations show, is not always the case.

The so-called nuclear sap is an important material, without the presence of which the homogeneous karyokinetic halves could not attain the

condition of nuclei. It is in preparation for the coming division, and for the purpose of accumulating this substance, that all the forces of the cell are concentrated, thus compelling a cessation of the *migratory* activity of the karyokinetic mass just before division. This accumulation of nuclear fluid is a process of nutrition, effected under the attractive influence of the nucleus, and the systems of rays are an evidence of the existence of this nutritive process. These solar figures have, after all, the same physiological significance, whether they are composed of stout or slender rays.

The last structure produced by the karyokinesis — the hollow cask — is composed of different elements. The fibres and rods divide directly, but the contents of the cask, which are not of a protoplasmic nature, are not capable of direct separation, owing to their viscidity; but during elongation fine granules, which are embraced by the cask, form themselves into filaments. These, however, are karyokinetic granules, which were not employed in the construction of the staves of the cask.

The last admission seems to me to remove all reasonable ground for a distinction between the peripheral and the central fibres of the cask-like stage of the nucleus.

New nuclei are formed, not by a differentiation of the homogeneous mass simply, but by a disintegration brought about by renewed motion. Notwithstanding the important influence of the nuclear sap in the formation of new nuclei out of the karyokinetic halves, the latter, remaining unchanged, are employed in the production of the nuclear *membrane* without experiencing the influence of the sap (!). The construction of the membrane out of rods and granules is the cause of its punctate appearance when seen in optical section.

The second division of FLEMMING's (**'78**[b]) paper deals with cell division in tissues, both normally growing and inflamed. In the division of the epithelial cells of the caudal fin and the gill-plates of Salamandra, he distinguishes a series of phases.*

Phase 1. There arises a "basket trestle" of closely coiled, exceedingly fine filaments, which gives the living nucleus the appearance of being finely granular, and as such it has usually been described. No evidence of nucleoli is to be found, but the nuclear figure has a sharp limitation toward the protoplasm. As compared with the network, or trestle, of the quiescent nucleus, it is much closer (i. e. has finer meshes), more evenly distributed through the substance of the nucleus, and represents a greater mass of substance. Is it directly connected in

* The order of their succession is considered later.

its origin with the trestle of the quiescent nucleus, or is it a new structure? In other words, Is there, on the one hand, a complete karyolysis (Auerbach), or a homogeneous condition (Strasburger)? or, on the other hand, is there no such stage intervening between the network of the quiescent nucleus and this finer trestle-work? If the former were true, then one should at least occasionally find evidence of the existence of entirely homogeneous nuclei. Such is not the case. Instead, one finds on carefully prepared objects nuclei presenting peculiarities which favor the latter view, and for the following reasons: — (*a.*) Such nuclei present various degrees in the sharpness, compactness, and fineness of the network. The finer and closer the network, the more uniform the thickness of the filaments and the greater the tendency (especially at the periphery) to a sinuous course. (*b.*) In the quiescent stage the intermediary substance (Zwischensubstanz) is capable of staining. In the definite glomerate stage (Knäuelstadium) there is no longer any "Zwischensubstanz" capable of being stained. Intermediate conditions, in which this substance has not all disappeared, correspond to intermediate stages in the formation of the characteristic glomerule. This change appears first at the periphery of the nucleus. Flemming, therefore, draws the following conclusion. The first metamorphosis of the nucleus in division consists in this, that the whole of its *stainable substance*, inclusive of that contained in the nucleoli and the nuclear membrane, is appropriated for the nuclear trestle, which thereby grows, at first becoming finer, and extends itself uniformly through the nuclear space in the form of meandering filaments; it therefore undergoes so complete a metamorphosis, that it is no longer comparable with the trestle of the quiescent nucleus.

Aside from these changes of the nucleus, the protoplasm of the cell also undergoes changes. (*a.*) The whole body of the cell passes from a flattened to a more nearly spherical form, — this, however, is principally due to a corresponding change in the shape of its nucleus, — and its outline often becomes more rounded. (*b.*) A more important change is an internal one, *which exists already in this first stage*, — a *dicentric arrangement* corresponding to the *future* poles of the nuclear figure.* The only

* I have italicized parts of this last sentence to call particular attention to the early appearance of this dicentric arrangement of granules in the protoplasm of tissue cells. I must grant, it is true, that Flemming furnishes no evidence that this dicentric arrangement *introduces* the changes of division, but that, on the contrary, it is the nucleus itself which first exhibits changes from the quiescent condition. It, however, will not be forgotten that Flemming's attention has naturally been concentrated on the remarkable alterations in the nuclear substance, whereby the possibility

evidence of this is found in the arrangement of pigment granules, fat drops, or (in the younger larval stages) remnants of the yolk granules, which in the quiet cell lie grouped uniformly around the nucleus or are quite irregularly distributed. "Wenn aber der Kern in die erste Theilungsphase tritt, *haben* sie sich zu zwei Gruppen *geordnet*." * These groups in oval cells usually correspond with the poles of the longer axis, the plane of division with the shorter axis; in some rare cases, however, this relation is reversed. When the division-axis of the cell chances to be very oblique to the plane of the object stage, the grouping of these "polar granules" is seen less in profile, and then exhibits a distinct radial arrangement (*loc. cit.*, Taf. XVI. Fig. 6 *a*).

Phase 2. The loose glomerate or basket form † of the mother nucleus. This more open-meshed condition implies a diminution in the number of the filaments, or, more probably, a diminution in the total length of *the* filament, and is accompanied by a corresponding increase in thickness, so that the volume of stainable substance remains the same as in the first phase. This thickening, it is believed, is not brought about by a direct fusion of neighboring filaments, — since no filaments are found which are in part of their course as slender as in the first phase, and in the adjoining part twice as thick, — but is due to a slow process, shortening the filaments, and at the same time making them correspondingly thicker, as in the contraction of a muscle fibre. This

of overlooking early changes in the protoplasm is greatly increased, and, what is of more importance, that these tissue-cells can hardly be claimed to be favorable objects for determining when the earliest appearance of a dicentric arrangement in the protoplasm takes place. I will not, however, insist here on the legitimacy of what I have only ventured to call attention to in another connection as seeming worthy of more careful attention before the initiative and controlling influence in cell division is definitely — not to say exclusively — assigned to the nucleus (or nuclear substance).

* The words here italicized are not so in the original.

† The basket form here spoken of is not identical with a condition of the nucleus thus named by Mayzel and Eberth. The structure intended by them is called by Flemming cask- or half-cask-shaped. It is only the *staves* of the cask and the *ribs* of the basket which are represented in these figures. Flemming recognizes the inaptness of the expression "basket-form" (previously employed by him) to represent this glomerate condition for the following reason: the glomerule of tortuous filaments, although it is more compact at the periphery than in the centre, is not simply limited to bounding a cavity. On this account he prefers the name glomerule (Knäuel), even though this is objectionable as implying that the thread is wound about a definite centre, which is really not the case.

If ravelled yarn be simply balled together *without winding*, the condition of the tortuous filaments will be very well reproduced; it would only remain to make the mass somewhat more compact at the periphery than in the centre.

process is, however, too slow to be directly observed in the living nucleus; only a series of successive drawings makes it apparent. The course of the filaments becomes more generally perpendicular to the major axis of the nucleus. Toward the end of this phase the sharp contour, which, in the first phase, marked the place of the nuclear membrane, is no longer present, but there is *a clear zone* around the nuclear figure, which is visible in *living*, as well as in hardened cells. This, although possibly more extensive on hardened preparations, is not, then, solely artificial (Mayzel). In preserved preparations delicate branched cords may be seen to connect the periphery of the nuclear figure with the cell plasm.

Phase 3. Star form of the mother nucleus. Just how this phase is produced from the preceding cannot be determined on living nuclei. Flemming believes it is accomplished through intermediate stages, the most characteristic of which is the *crown form.* In the latter the course of the filaments is almost exclusively *radial*, and in many cases a central space is left entirely free; both at the periphery and near the centre the filaments form *loops*, and thus show that they are more or less continuous. This hollow sphere of looped filaments gradually affects an arrangement in a single plane (not that of the approaching division), and therefore is more directly comparable with a ring, or, if the centre is not hollow, with a disk. The *peripheral* loops of this crown now break through, so that there are twice as many free ends as there formerly were peripheral folds. The rupture need not necessarily be at the apex of the loop. Thus the crown form passes into the star form. Already in this stage a *dicentric grouping* is sometimes to be observed in the *stellar figure,* since the loops which correspond to the division poles are the *first* to be ruptured, those near the equator the last. Very rarely there is a genuine *double star* with completely separated centres.*

Another remarkable change takes place either at the end of the previous, or during the present stage. The *filaments split lengthwise each into two*. This is quite typical, at least for Salamandra. After the rupture of the peripheral loops the free ends of the split filaments diverge, and thus give rise to a *fine-rayed star.* This fission of the nuclear filaments is not the result of the use of reagents, as it has been seen in the living cell also. It has been observed to remain in this condition for two hours, and meantime the stellar figure undergoes a series of slow changes of form — a sort of systole and diastole — which affects the

* This separation, I would add, is not to be mistaken as equivalent to the division of the equatorial plate.

polar rays much more than those of the equator. These oscillations have the effect of giving the figure at the end of the systole the form of a spheroidal body, with flattenings, or even funnel-shaped depressions, at the poles.

Phase 4 is of short duration, and is characterized by the formation of an *equatorial plate*, during which the filament elements become grouped parallel to the axis which is perpendicular to the plane of division. The thickness of the plate is from one fifth to one third as great as the whole length of the cell. At first, however, it is only the filaments in the axial portion of the figure which are parallel, or slightly converging toward the poles; the more peripheral are still tortuous. But this "equatorial-plate" stage, which recalls the "nuclear spindle" of other authors, differs in several points from the typical spindle. The equatorial thickenings known collectively as the "Kernplatte" are wanting here,* and the filaments terminate *at the equator in free ends*. Whether or not this equatorial interruption in their course is induced by an immediately preceding division, it is difficult to say. Certain preparations which show a continuity still existing between *some* of the fibres belonging to the opposite halves of the figure favor the view that they were recently continuous throughout, and yet this appearance may have been brought about by a temporary apposition of filaments which are really distinct, and were actually separated into two groups at an earlier stage. The occasional existence of *double stars* during the preceding phase strengthens the latter hypothesis; for, if the substance of the daughter nuclei in these cases was already segregated in such a manner that each star embraced all the nuclear substance destined for the nucleus of its own pole, then a temporary apposition of the filaments must necessarily be predicated. But if the double-star condition does not normally come in the series of changes, it is possible that a separation of the nuclear halves may have transpired before the equatorial-plate stage. In this connection Flemming explains how the longitudinal fission of the filaments may possibly be equivalent to such a separation, one half of each filament going to one of the new nuclei, the other half to the other. This stage may possibly be comparable with Mayzel's spindle with outlying nuclear plate (see p. 348). Continuous observation of this "nuclear-cask" stage shows that the cask becomes broader, and that its peripheral filaments become alternately curved and straightened at the equatorial end.

Phase 5. Separation of the halves of the nuclear figure. This is also of short duration. Each half has the form of a broad fish-weir.†

* See also Mayzel for Triton.

† This is the basket stage of Eberth.

The equatorial interval between the free ends of the filaments increases. In the case of Salamandra there is something, like the "Kernfäden" of Strasburger, occasionally left stretched between the separating filaments, but they are not continuations of the substance of which the nuclear filaments are composed, for they *are not stainable.*

Phase 6. Star form of the daughter nuclei. The filaments of each half of the nucleus — till now directed toward the equator of the cell — begin to spread out so as to lie more nearly parallel with the equatorial plane, and thus a somewhat flattened starlike figure is produced. The axis of division (i. e. the line perpendicular to the centre of the plane of division) now becomes curved, so that one or the other of these stars is seen more *en face,* and then exhibits a free central space. Indications of constriction often appear at this time on one side of the cell.

Phase 7. Crown form, and glomerule form of daughter nuclei. In this, which resembles Phase 2 (mother nucleus), the two crown-shaped nuclear masses have a somewhat flattened concavo-convex form, the *convexities* being directed toward the equator. In this phase the cell is divided by a continuous, constantly advancing constriction, and *without any differentiation within the cell in the equatorial plane.* That is to say, there is no evidence of a "Zellplatte." The central and subcentral portions of the protoplasm are comparatively passive in this cell division. If there is a "contraction," it is to be located in the cortical layer of the protoplasm. If it is either wholly or in part a matter of attraction in totality (Gesammtattraction) toward polar centres, then it must be said that the grouping (readjustment) takes place so imperceptibly that its progress finds distinct expression only at the periphery.

Phase 8 ("if such is to be distinguished"). Trestle form of daughter nuclei, and reversion of the same to the quiescent condition. During this phase the nuclear filaments assume a direction perpendicular to the long axis of the nucleus, and from that pass to a uniformly disposed trestle in which the filaments are no longer curled. This becomes more and more compact, and at the same time paler, while it increases in size. About the beginning of this phase it also becomes sharply limited from the cell plasm, and the intermediary substance becomes stainable. A veritable membrane appears only after the trestle of uniformly disposed elements has come into existence, and, as he thinks, is probably formed by a fusion of the peripheral filaments of that trestle. The origin of nucleoli was not discovered.

From these latter phases it is clear that the daughter nuclei have at first a flattened stellate form; that this passes into that of a crown of

curled filaments, which are continuous by means of central and peripheral loops; that from the latter arises a meandering glomerule (Windungsknäuel) and from this a trestle with intermediary substance. Aside from the double star *this is the same series of forms assumed by the mother nucleus, but in reverse order.*

From a comparison of the whole series of changes Flemming presents the following scheme as probably representing the double series of nuclear changes which accompany cell division in Salamandra, — the one progressive and affecting the mother nucleus, the other regressive, and (since it follows the division) pertaining to the daughter nuclei.

Mother nucleus (progressive).	*Daughter nuclei* (regressive).
1. Trestle (quiescent).	1. Trestle (quiescent).
↓	↑
2. Fine-thread glomerule.	2. Fine-thread glomerule.
	↑
3. Thickening of the fine threads and loosening of the coils.	3. Narrowing [of the coils].
↓	↑
4. Central and peripheral loops (crown form).	4. Central and peripheral loops (crown form).
↓	↑
(Rupture of the loops.)	(Union into loops ?)
5. Star form of mother nucleus.	5. { Star form of daughter nuclei. Coarse-rayed half-cask.
↓	↑
6. Fission of its rays.	6. Fusion of rays in pairs (?).
↓	↑
7. Fine-rayed star.	7. Fine-rayed, half-cask.
↘	↗

8. Equatorial plate.

Results on other cells are mostly confirmatory of the changes given above. However, in connective-tissue cells an incomplete division results in a cell having two nuclei; this is more rarely seen in epithelium. In red blood-corpuscles the nuclear filaments so increase in extent as to reach to near the periphery of the cell. The author thinks the cell substance has in this case contributed to the remarkable increase in the bulk of the nuclear figure, and finds an argument to support this view in the fact that filaments of the nuclear figure in *unstained* chromic acid preparations have a peculiar greenish brown or brownish yellow color corresponding to that of hæmoglobin. This, he adds, would be a striking confirmation of Auerbach's theory of the mingling of nuclear substance and cell substance in division, were it not that the substance of blood-cells is very peculiar as compared with that of other cells, and that in other cells such a phenomenon does not take place.

The points in the metamorphosis of the nucleus to which Flemming has here called attention for the first time are: — (1.) the first stage, which is not granular, but presents a connected system of curled, very fine filaments; (2.) the delicate glomerule stage which immediately follows; (3.) the loosening in the coils; (4.) the stage with peripheral and central loops; (5.) the rupture of the loops to form a star; (6.) the fission of the filaments; (7.) a bipolar arrangement of the figure during a series of rhythmical contractions and expansions; (8.) the fact that the daughter nuclei do not at once form a homogeneous mass, but pass in reverse order through the stages which the parent nucleus undergoes during the metamorphosis.

The objections which Auerbach ('76) has raised against identifying the spindle- or nuclear-figure with the mother nucleus are answered by Flemming point by point: — (1.) the nuclear figure is not always larger than the quiescent nucleus, and in cases where it is larger the old nucleus has already before division so increased in size that the masses of both nearly agree; (2.) the sharp nuclear membrane, it is true, is lost, but the nuclear figure still remains sharply limited from the cell plasm; (3.) there is no stage found in Salamandra where the old nucleus has actually or apparently disappeared, but the nuclear figure is morphologically derived from it; (4.) the new nuclei *do* arise from a division of the old nucleus. It remains, however, to ask, says the author, whether in the formation of the nuclear figure anything is taken from the protoplasm, whether anything of the substance of the nucleus goes at this time to the protoplasm, and, finally, if any like change takes place during the growth of the new nuclei. All three suppositions are possible. It is certain that the clear substance between the curled filaments does not directly go to the new nuclei. One cannot, then, hold to a complete identity of the nuclear figure with the nucleus. In red blood-corpuscles a large part of the cell substance, in fact, almost the whole of it, is incorporated in the division figure. If this is not the case in other cells, it can hardly be denied that a small part of the substance of the nuclear figure is annexed from the protoplasm. An exchange between nuclear substance and plasma as a general phenomenon is, if not proved, at least to be assumed as possible. Fol, Auerbach, and the author himself, says Flemming, have rightly maintained that a cell division in Remak's sense does not cover the facts, but they have as certainly fallen into error in maintaining that no formal element of the nucleus remains.*

* "Dass ein solcher Kern bei der Theilung nicht bestehen bleibt (i. e. as membrane, contents, and nucleoli) und sich *nicht* direct entzweischnürt, dass also die alte,

Flemming dissents from the general views expressed by Strasburger ('76 and '77) as follows: —

(1.) The nucleus is not always elongated at the beginning of division.

(2.) It is not homogeneous before division.

(3.) A polar opposition at two peripheral points of the nucleus is formed in the "Anfangsstadien," as is shown by the grouping of the "polar granules" in the plasma; but this polarity does not *here* (Salamandra) come to the morphological expression in the *nuclear mass itself*, which Strasburger lays down as a general principle. The poles, in these animal cells, are *not* characterized by a special refractive power. "*Während die Polkörner schon lange gruppirt sind, besteht noch keine dicentrische Ordnung in der Kernfigur.*" There follows, rather, a radial (monocentric) grouping — the star form — which finds no place in Strasburger's scheme. At least, there is no visible expression of an accumulation of part of the active nuclear substance at the poles, and a repulsion of another part toward the equatorial plane, in this stage, nor yet in that of the formation of the "equatorial plate" (Flemming), inasmuch as at this time the whole of the substance to become new nuclei is collected at the equator.

I have given in some detail Flemming's views in this connection, because they seem to confirm in a very decided way certain of the conclusions at which I had already arrived from the study of entirely different objects, and on somewhat different grounds. It cannot fail to impress one as rather remarkable that there should be no evidence of a bipolar condition in the *nucleus* when the *protoplasm* was already thus distinguished, if, as has generally been believed, it is *nuclear substance*, which constitutes the centres about which the dicentric arrangement finds expression. Evidently the tissue cells, which afforded the material for his study, are not the most favorable objects for determining the exact position of the centres about which this polar grouping of plasma granules takes place. Perhaps the eggs of Limax may help to explain why this early grouping ensues. I am inclined, in view of the possibility that the centres of attraction may be *at first* phenomena of the cell plasm and not of the nucleus, to suggest that these centres may also in *tissue* cells lie totally outside the nucleus, as differentiations in the protoplasm. I attach little weight to the supposed absence of a special refractive power of the poles, since, for two reasons, it may have been overlooked; namely,

Remak'sche Lehre von der Zelltheilung nicht zutrifft, darauf haben Fol, ich und Auerbach mit vollem Recht aufmerksam gemacht; und mit ebenso vollkommen Unrecht haben wir angenommen, dass vom Kern wirklich nichts Geformtes restire, weil es an unseren Objecten nicht zu sehen war."

the unfavorable nature of his objects for determining the exact position of the centres of attraction, — for the granules are very scanty and of comparatively large size, — and the fact that he was especially interested in a confirmation (or negation) of Strasburger's view, whereby his attention was directed to an accumulation of active "*Kern*stoff" in this vicinity.

The evidence to be gathered from Flemming's paper as to the exact condition of the nuclear membrane when these polar figures of the protoplasm *first appear*, is not absolutely decisive in favor of the view that the centres of attraction are formed quite independently of nuclear substance, although his own conclusions are positive (*loc. cit.*, p. 364) in excluding the possibility of a mingling of nuclear mass and protoplasm at this stage.* The fact that the dicentric arrangement in the protoplasm is not shown on the figures of stained preparations (except at a much later stage) makes it the more difficult to form a just idea of the temporal relation of these two series of phenomena, — the plasmic and the nuclear. However, Flemming assures us (in the explanation of the plates) that the stage reproduced in Taf. XVI. Fig. 2 *a* (where the appearance of the dicentric arrangement is *first* figured) corresponds to the stained specimen (Taf. XVII. Fig. 3) to which the description just pointed out refers. This seems to indicate the same conclusion as must be drawn from Fig. 52 of Limax, where the nuclear membranes of both pronuclei are still *intact*, *sharply defined*, *double-contoured structures*, and where one of the polar stars has already made its appearance. It is certainly difficult to conceive how any *formed* substance could, in this case, have come to occupy such a position outside the nuclear membrane. I cite this figure for two reasons: the egg was hardened in chromic acid (not in acetic acid, which has been thought to produce an artificial appearance of the nuclear membrane), and the sections were all preserved, so that I can say with positiveness that only *one* stellar figure was recognizable in this case. This is of importance in showing a closer approximation to the *first* appearance of the stellar figure than most other observers have made, and therefore serves to justify my conviction that their conclusions may, at least in some cases, rest upon insufficient observations.

* "Dagegen besteht noch jetzt eine scharfe Abgrenzung der Kernfiguren gegen das Plasma, allerdings nur an gefärbten Objecten (Taf. XVII. Fig. 3) sicher zu stellen als ein feiner, aber scharfer Contour. Derselbe kann aber mit der alten Kernmembran nicht mehr identisch genannt werden, denn er ist zarter und nicht immer so deutlich, wie sie, tingirbar; vielleicht ist er ein Rest von ihr, vielleicht nur der Ausdruck der Grenze zwischen Kernmasse und Plasma, — *aber es ist Gewicht darauf zu legen, dass diese Grenze auch noch in diesem Stadium eine scharfe, eine Vermischung von Kernmasse und Plasma also für dasselbe noch jedenfalls auszuschliessen ist.*"

Flemming finds in his studies nothing to homologize with the "Kernspindel," and therefore concludes that division may take place in the cells of animal tissues without such a structure. In Triton and Salamandra nothing of the interzonal filaments (Kernfäden, Strasburger) or the cell plate has been discovered. So, too, the thick fibres which Strasburger calls in Nothoscordum fragrans "Kernspindel" are really homologous to the whole stainable nuclear figure, and are therefore comparable with "Kernplattenelemente" rather than the spindle.

In view of Peremeschko's statement that in Triton there are equatorial thickenings in the cask-shaped structure, and of Schleicher's recognition of interzonal filaments, these conclusions of Flemming are less convincing. One may still entertain a doubt if, after all, we should not recognize in some of the stages seen by Flemming (e. g. Taf. XVII. Fig. 14) the equivalent of spindle and nuclear plate combined.

Flemming emphasizes the fact that the stellate or monocentric condition of the *nuclear substance* is not to be confounded with a stellate figure of the *cell* plasm, and yet that the two are so similar that it would seem extremely improbable that they were such merely by chance. He says, "It seems to me hardly deniable that the yolk radiations as well as the stars represent a visible expression of the forces which at that particular time are active in the cell substance and nuclear substance, and which operate according to a *mono*centric-radial type before the division, a *di*centric-radial type after the division." (*loc. cit.*, p. 422.)

It does not seem to me that his own observations justify a conclusion which makes the forces effecting both these conditions identical, and the figures themselves the *successive* expressions of the same continuous force. Viewing the cell as a whole, they are not simply *successive* phenomena; they are co-existing. The changes in the *nuclear substance* lead from the monocentric to the dicentric condition, and so far there is a succession, in the manner suggested; but with the cell as a whole it seems much more as though there were *two distinct* (though analogous) and in a sense *antagonistic forces*, — one acting from the *centre of the nuclear substance* and finding expression in the monocentric condition of the same; the other acting from the stellar poles in the *cell plasm* and ultimately dominating, till, with the effected division, the first or nuclear force is enabled to reassert its supremacy. In this connection I must repeat what has been so many times said, that the centre of the new nucleus (the seat of the *nuclear* force) is not identical in position with the centre from which the forces of the cell plasm operate. It is possible that this hypothesis may account for the oscillations (systole and diastole) observed

by Flemming to take place in the nuclear substance; for these oscillations occur at a time when the monocentric may be supposed to be giving way to the dicentric attraction.

γ. Plants. — A part of the spindle metamorphosis of the nucleus, — namely, the equatorial plate, — appears to have been seen at an earlier date in the division of vegetable cells * than in that of animal cells. The real significance of the disk was overlooked, however, in the belief that its components were artificial products, — alterations produced in the albuminous fluid of the middle of the cell by the protracted action of water.

Russow (**'72**) was the first to correct this mistake, and to establish, in the year succeeding the appearance of Kowalevsky's paper, and quite independently of his discovery, the normal existence of granular zones in certain plant cells similar to those seen by Kowalevsky. These were the parent cells of spore and pollen elements. The observations in the case of ferns were made only after a study of the less obscure structure in Ophioglossum and Equisetum. It was in these that the nuclear plate (Kernplatte Strasburger) was for the first time accurately observed, but the less conspicuous spindle fibres were not seen. The relation of the plate to the nucleus was, however, very cogently argued. The separated halves of this nuclear disk were also seen, but not fully understood, nor their mutual recession suspected. It was pointed out that the *nuclear* plate, and the structure afterwards called by Strasburger *cell* plate, were not identical, so that what is said (*loc. cit.*, p. 51) concerning the "Körnerplatte" (cell plate) in the formation of spores in Marsilia is in no sense to be referred to the nuclear structure mentioned.

Russow's statements (pp. 89, 90) are as follows: "Neben Mutterzellen von dem geschilderten Aussehen [i. e. with a very large, finely granular, spherical nucleus, usually eccentric in position], findet man (bei Polypod. vulgare und Aspid. Filix mas) andere, die statt des Kerns eine kreisförmige Platte von $\frac{1}{2}$ bis $\frac{2}{3}$ Durchmesser der Mutterzelle führen, deren Fläche grob granulirt, deren Rand, wenn man auf denselben nach Drehung der Platte um 90° herab sieht, wie aus länglichen Körnchen, oder kurzen Stäbchen, die hell und stark lichtbrechend sind, zusammengesetzt erscheint. Grösser und schärfer ausgeprägt sind diese Körnchen- oder Stäbchenplatten in den Sporenmutterzellen der Ophioglosseen und Equisetaceen (Figs. 121, 122, 123, 126); am grössesten und in ihrem Bau am deutlichsten erkennbar fand ich die Platten in den Pollenmutterzellen

* Hofmeister, Die Lehre von der Pflanzenzelle (1867, p. 82, Figs. *d* and *e*). See also the explanation of the figures.

von Lilium bulbiferum (Fig. 132); hier bestehen sie aus kurzen, verhältnissmässig dicken, wurmförmigen Körperchen oder schwach gekrümmten Stäbchen, die farblos, hell und matt glänzend sind, und durch Jod kaum merklich gefärbt, durch Alcalien (selbst bei grosser Verdünnung) carminsaures Ammoniak und Chlorzinkjod fast momentan aufgelöst werden, ohne sich zu färben. Dasselbe chemische Verhalten zeigen die Platten bei den Ophioglosseen, Equisetaceen und Farnen.

"Diese Stäbchenplatten . . . . sind durchaus verschieden von den s. g. Körnerplatten oder Protoplasmaplatten, die nach dem der primäre Kern geschwunden und 2 neue (secundäre) Kerne erscheinen, zwischen letzteren auftretend die Sporenmutterzelle halbiren, oder, die nach dem Erscheinen der 4 tertiären Kerne auftreten, um die Sporenmutterzellwände zu bilden. Aus dem Umstande, dass zur Zeit, wo Stäbchenplatten vorhanden, nie Kerne sichtbar sind, und dass . . . . nach dem Auftreten der die Mutterzelle halbirenden Körnerplatte zu beiden Seiten letzterer, wo sonst die Kerne vorhanden, je eine Stäbchenplatte (von dem halben Durchmesser der primären Stäbchenplatte) sichtbar ist, darf man wol auf eine nahe Beziehung zwischen Kern und Stäbchenplatte schliessen, wenn nicht auf die Bildung letzterer aus ersterem. Bei Polypod. vulgare und Aspid. Filix mas habe ich nur in wenigen Fällen nach dem Auftreten der Körnerplatte zu beiden Seiten derselben Stäbchenplatten wahrgenommen, doch glaube ich, dass ihr Auftreten hier wie in den Sporenmutterzellen der Farne und Gefässkryptogamen überhaupt und wahrscheinlich auch in den Pollenmutterzellen der Phanerogamen eine regelmässige Erscheinung ist, die nur in den meisten Fällen wegen ihrer Kleinheit und anderer ungünstigen Umstände halber sich der Beobachtung entzieht."

The following is from the detailed description of the Stäbchenplatte in the case of Ophioglossum vulgatum (pp. 126, 127): "Die Stäbchenplatten sind selten ganz eben, meist ein wenig gewölbt oder am Rande verbogen; am besten erkennt man ihren Bau, wenn ihre Fläche zu der Sehaxe des Auges ein wenig geneigt steht; werden sie durch einen ziemlich starken Druck aufs Deckglass alterirt, so tritt ihre Zusammensetzung aus verbogenen Stäbchen oder wurmförmigen Körperchen* besonders deutlich hervor."

The succeeding changes are: (1.) the disappearance of this primary disk (Stäbchenplatte); (2.) the appearance of two (secondary) nuclei of about half the size of the primary nucleus; (3.) the appearance of a "Körnerplatte" (cell plate) between these; (4.) the appearance of two

* Compare pp. 355 – 362 (Flemming).

(secondary) disks in place of the nuclei, smaller than the primary disk; (5.) the disappearance of the secondary disks; (6.) the appearance of four (tertiary) nuclei in positions corresponding to the four angles of a tetrahedron.

Of the secondary disks, it is further said that their surfaces are either (*a*) parallel with, or (*b*) perpendicular to, the Körnerplatte, from which it may be justly inferred that the author mistook in the first case (*a*) the mutually receding lateral halves of the *primary* disk for secondary disks, and that only those having the position indicated under (*b*) were really secondary structures. He says concerning the latter that they may lie in the same plane, or their planes may be mutually perpendicular. The latter arrangement evidently corresponds to the tetrahedral disposition of the four nuclei, which result from the division of these two disks.

In the Equisetaceæ (p. 148) "numerous stages of transition between these disks and the spherical cell-nucleus, with respect to the size of the whole structure as well as the rods (Körperchen) which compose it, are observable."

As Strasburger has already pointed out, Tschistiakoff ('75) was the first to observe in plant cells the fibrous differentiation of the nuclear spindle. The account which he (*loc. cit.*, col. 20) gives for the mother cells of the microspores of Isoëtes Durieui, is essentially the same as that for Lycopodium and Equisetum (col. 24, 25). In Angiopteris (col. 7), where the spindle figure was not observed, the protoplasm during the division of the cell is entirely homogeneous, without any such morphological differentiation as nucleus and nucleolus. Inasmuch as these *are* visible when the cell is subjected to the influence of water, he offers the following as an explanation of the phenomena. The plasma undergoes several steps of *chemical* metamorphosis, which begin at the centre of the cell contents. The inner plasm absorbs more water than the more peripheral portions, because it is in a more advanced state of chemical metamorphosis, and thereby the optical properties of the parts become so different that the inner one becomes distinguishable as a nucleus, whereas before the action of the water it had no definite boundaries. Therefore, nucleus, nucleolus, and primordial utricle are only so many successive steps in the process of metamorphosis.*

This seems to be very nearly equivalent to saying they have no *mor-*

* See also Tschistiakoff, Notice préliminaire sur l'histoire du développement des sporanges et des spores de l'Isoëtes Durieui, Bory. In Nuovo Giornale Botanico Italiano, Tom. V. p. 207.

phological importance! For this indistinguishable "physiological nucleus" Tschistiakoff employs the term *pronucleus.*

In Isoëtes this "pronucleus," under the influence of water, exhibits, according to Tschistiakoff (col. 20), the form of an ellipsoid, or, rather, appears to be composed of two cones placed base to base; the long axis corresponds with that of the cell; the substance of the pronucleus is differentiated, so that one sees upon its surface more or less compact lustrous streaks, which are arranged as meridians. These streaks ("Differencialia") are apparently spindle fibres, and his "pronucleus striatus" is the so-called spindle figure, or nuclear spindle. In a more advanced stage, he continues, an equatorial welt of still more compact substance is to be observed on the surface of the "pronucleus." This is simply a plasmatic plate composed of projecting papillæ, through which the plasm is divided in its physiological centre. At this time there are two small protoplasmic spheres near the poles of the "pronucleus" [central areas of asters?] which are soon converted into small vacuoles [the equivalents of Bütschli's multiple nuclei?], — the "pronuclei" of the two new parts. The plate which divides the "pronucleus" appears in the form of a welt, because it is more compact than the rest of the "pronucleus." This structure subsequently extends to the other parts of the protoplasm; the division proceeds from the centre toward the periphery. The division of the protoplasm ensues in consequence of the molecules becoming grouped according to their polarity. From this it is evident that the groups, being in nature alike, must separate by reason of the force of mutual repulsion.

The formation of *macrospores* out of the mother cell in the case of Isoëtes is similar to the formation of spores of Anthoceros. The mother cell has a *morphological* nucleus. The groups of starch granules are not held, as formerly,* to be nuclei; the secondary nuclei are not formed in the presence of the primary nucleus, although the masses of starch are. In the vicinity of the latter, innumerable transparent protoplasmic filaments diverge in all directions. By the crossing of these filaments in the middle of the cell there arise compact uniform plates [cell plates], which, like the starch granules, have a tetrahedral arrangement, and which serve, by their fission, to divide the cell into four portions. Four secondary nuclei are found in the centre of the mother cell, each corresponding to a cluster of starch granules. They are, however, much nearer the planes of division, and are formed *in the interior* of the primary nucleus, which is subsequently dissolved.

* See Nuovo Giornale Botanico Italiano, Tom. V.

This differs in several particulars from the more recent account given by Strasburger.

In pollen mother-cells of Magnolia and Conifera a nuclear spindle has also been seen by Tschistiakoff; but the description is so confused through the introduction of imaginary "pronuclei" and "pronucleoli" as not to be easily understood. In Magnolia, the equator and the poles of the "pronucleus" become more compact. The equatorial lamella becomes broader, and exhibits a meridional striation. The substance of the two poles represents the two prospective "pronuclei," during the enlargement of which the striation becomes indistinct. Each of the two resulting secondary "pronuclei" possesses two nucleoli. Each of the "pronuclei" now divides, and so quickly that the act is almost simultaneous with the first cell division.

In conifers after the nucleus has again assumed the condition of a "pronucleus" there appear either one or six very fine protoplasmic division-lamellæ. This is an indication of the division of the "pronucleus" either into two parts, or at once into four, which then have the tetrahedral arrangement. Furthermore, both "pronucleus" and "pronucleolus" are very peculiarly streaked upon the surface by a multitude of serpentine lines consisting of more compact and lustrous portions of the pronuclear substance.* These lines become changed into broad, compact, brilliant bands in the form of projecting meridians. The equatorial division-plate of lustrous protoplasmic corpuscles becomes broader. Two protoplasmic regions, representing the prospective secondary nuclei, now make their appearance at the poles. The streaked equatorial zone persists for some time, but finally the streaks disappear, and the two new pronuclei are separated by a wide zone of protoplasm. The "pronuclei" are soon converted into nuclei.

It will be seen that Tschistiakoff has not made a sharp distinction in his description between cell plate and nuclear plate, and that the broadening of the equatorial zone is the only thing that even hints at a migration of the halves of the nuclear plate.

The criticisms of AUERBACH (**'76**[a]) are directed toward the views expressed by Strasburger in the first edition of his celebrated work, "Ueber Zellbildung und Zelltheilung." As that edition has not been formally considered in the present paper, the reader is referred to the review of the second edition, to be found at pp. 372 *et seq.* Auerbach has now observed that the middle piece of his karyolytic figure is more spindle-shaped than previously (1874) represented by him, and that it has a

* Compare Flemming's (**'78**) descriptions of the nuclei of animal tissue-cells.

meridional striation; but this, he thinks, in no way invalidates his previously expressed views.

In two points he dissents from Strasburger's conclusions: (1.) the process of the neoformation (Neubildung) of nuclei; (2.) the method of their increase. He maintains that the nucleus is at first a sort of vacuole, — a droplike accumulation of a "dickflüssig," clear, homogeneous substance, in a cavity of the protoplasm, which has at first no special limiting layer. Subsequently, the protoplasm in immediate contact with the surface of this nuclear drop becomes compacted into a special "Wandung," — the nuclear membrane, — and one or several nucleoli are formed by a gradual agglomeration of finest spherules. He believes Strasburger's view — that the nucleus is only a more or less sharply segregated portion of the cell protoplasm — rests upon a misconception of the true nature of the structures which he has called "cell" and "nucleus" in the endosperm cells especially of Phaseolus multiflorus. According to Auerbach these are respectively *nucleus* and *nucleolus*. This revision of Strasburger's conclusions he endeavors to substantiate by an examination of the properties of the structures in question. In the first place Strasburger's "nuclei" are typical *nucleoli*, which, in small nuclei, are always dark, solid spherules in the centre of the nuclear space, and which undergo the changes ascribed by Strasburger to his "nuclei," — becoming often irregularly pointed and vacuolated, whereas *nuclei* are uniformly clear bodies in dark protoplasmic surroundings. Again, his "cell" cannot be a cell, since from the beginning it is a vesicle (Hohlbläschen), whereas a free-formed cell is never a vesicle at first. The radial appearance and netlike structure of this "cell" are not *necessarily* to be homologized with the netlike distribution of protoplasm so common in plant cells, since the same morphological condition is also known to exist in the *nucleus* of many (animal) cells.* Finally, there exists between these free-formed "cells" portions of the protoplasm of the mother cell. If the wall of the vesicle is the "Hautschicht" of a cell, one must assume that this protoplasmic mass, in which the cellulose membrane is formed, intervenes between the Hautschicht on the one hand and the cellulose membrane on the other; but that would be altogether anomalous.

The views here expressed concerning nuclear division are substan-

* Auerbach does not consider the network in this case to be composed of the same substance as nucleolus and nuclear membrane; instead of being nucleolar substance, it is of the same material which in other nuclei makes its appearance in the form of discrete spherules, — his so-called Zwischenkügelchen. Compare Auerbach '74.

tially a repetition of those already given in another paper. See pp. 304, 305. In addition, he maintains (p. 22) in regard to the spindle that two things have been confounded. A portion of the meridional lines are only rows of dark granules imbedded in the cell protoplasm, which lie at the *surface* of the spindle in the territory of the radial expanse of the karyolytic figure. With the use of a low magnifying power, or after employing hardening reagents, the yolk granules which are closely packed in the intervals between the rays that stretch from pole to pole (spindle) may have the appearance of continuous meridional lines.

I do not believe the filaments of the spindle in Limax can be accounted for in this way. There are no interfilamentous rows of protoplasmic granules in the territory of the spindle, but the spindle fibres themselves are, if not in the beginning, eventually much thicker than the extra-spindle rays.

Auerbach, for the sake of brevity, would substitute "Karyolyma" for "karyolytische Figur."

Incidental to a criticism of Tschistiakoff's use of "pronucleus" for the physiological nucleus, which subsequently becomes a *morphological* nucleus, he suggests that the at times apparently striate middle portion of the karyolyma may better be called *internucleus*. This, however, seems to rest on a misconception of, or refusal to recognize, the essential nature of the nuclear disk and its separated halves.

Among botanists, it is STRASBURGER ('76) to whom are due the most extensive contributions in this line of research.

Led, by the study of alcoholic preparations of embryos of the pine family, to the conclusion that before cell division the nucleus undergoes radical morphological changes, he successfully endeavored to control his observations by the close study of some living object on which the *inferred* metamorphoses might be followed step by step. He had come to the conclusion, from the study of alcoholic specimens of successive stages, that the nucleus before cell division becomes elongated, more or less ellipsoidal, and presents in its equator a peculiar plate composed of a single layer of nearly parallel rodlike granules; that, further, to both sides of this plate bands (Streifen) are attached, which converge toward the poles of the nucleus, thus lending to the latter a spindle-like appearance; that subsequently the plate of rods (Stäbchenplatte) becomes split into halves which, by mutually receding, approach the poles of the nucleus, but leave stretched between them numerous fine *Kernfäden* [interzonal filaments]. The substance of the halves after migrating toward the poles of the spindle forms two new nuclei, one for each of

the two daughter cells (pp. 26, 27). Such was Strasburger's conclusion, when he undertook its confirmation by the study of cell division on the fresh-water alga, Spirogyra. His results (pp. 34 – 37, 42 – 48) may be summarized as follows.

In the normal quiescent condition of the Spirogyra cell, the nucleus appears fusiform, with its axis perpendicular to the axis of the alga filaments.* The first change in a cell about to undergo division is a thickening of the nucleus. This is accompanied by a commotion in the enveloping granular protoplasm. The latter stretches out from the ends of the now cylindrical nucleus in the form of suspensory filaments. At length the nucleus has increased its thickness fourfold, and its nucleolus, at first increased in size, has entirely disappeared. Suddenly, after the solution of the nucleolus, the substance of the nucleus exhibits a filamentous differentiation, which proceeds from the lateral surfaces toward the equatorial plane. At the same time it becomes condensed in the equatorial plane into a highly refractive plate (Kernplatte). This central plate exhibits no structural differentiation in the fresh condition, but in alcoholic preparations it shows a continuation of the parallel striations of the lateral halves of the nucleus; but in the middle the bands are much thicker, and appear like short rods, which are separated by intervals equal to their own thickness. The plate is disk-shaped and reaches to the periphery of the nuclear mass. The whole striated nuclear structure is surrounded by a hollow cylinder of finely granular protoplasm, leaving exposed only the ends of the nucleus. The insertion of the nuclear filaments (Kernfaden), as seen from the end of the cylinder, embraces a circular area, and presents a finely stippled appearance. Simultaneously with these nuclear changes the first evidence of the approaching division appears in the mural protoplasm of the cell.

By further changes this striate nuclear structure becomes elongated in a direction corresponding to the length of the filaments, with accompanying decrease of diameter, and assumes the shape of a cask. Granular protoplasm collects at the ends of the cask-shaped nucleus; the thickness of the nuclear disk becomes increased by the lengthening of its component rods, each of which now shows a median constriction. In this way the nuclear plate begins to divide into lateral halves (Plattensegmente). These plate-segments separate so rapidily that the motion may be directly observed with a magnifying power of 600 diameters. In the separation the halves of the component rods move apart, but drawn-out filaments [interzonal filaments] of their substance serve still to unite their

* It in reality has the form of a biconvex lens.

swollen extremities. The whole cask-shaped nucleus undergoes a lengthening meantime, so that the two segments have made only comparatively little advance toward the ends of the nuclear structure. Both the nucleus and the surrounding protoplasm are in great commotion, and the latter is often radially disposed at the ends of the nucleus (p. 44). There appears a granular accumulation in the equatorial plane of the [interzonal] filaments, and into this median zone the latter are at length absorbed. About this time the protoplasm, which ensheathed the sides of the nucleus, is differentiated into a few (ca. 15) filaments [*not nuclear* filaments !], which are attached behind the disks (i. e. on their polar faces) in a circle.

When the interzonal filaments disappear, the granules of the lateral segments begin to fuse with each other and with the striated nuclear substance (nuclear fibres) which still remains between this structure and the ends of the lengthened cask-shaped nucleus. Thus solid disks are formed. The latter soon move into contact with the granular protoplasm which covers the ends of the cask-shaped structure. Each of the extranuclear filaments * soon presents at both its extremities — in the granular protoplasm covering the polar surfaces of the nuclear disks — a little swelling, and at the same time the course of the filaments becomes more convex outwardly. The equatorial granular accumulations of the interzonal filaments are ultimately transferred to these extranuclear filaments, and they in turn unite with the ingrowing girdle of mural protoplasm. Meantime the equatorial faces of the homogeneous solid disks become convex; there soon appears in each disk a few (2 – 4) highly refractive globular bodies, all but one of which are gradually dissolved and disappear; this one increases in size and becomes the nucleolus; it eventually comes to occupy the centre of the nucleus (disk), both faces of the latter having meantime become convex. By the distribution of the granular protoplasm over the whole surface of the two new nuclei, the latter are in all essentials like the nucleus from which they were derived.

With this more detailed account for Spirogyra the other studies of Strasburger on plant cells may be summarized by considering some of their deviations from the case just reviewed. Besides algæ the cells of various higher plants were studied, principally by means of alcoholic preparations. Stomatic and endosperm cells, the parent cells of pollen and spores, the hairs of Tradescantia stamens, etc., exhibited essentially

* By this name I would designate those filaments which are formed from the protoplasm lying outside the cask-shaped nucleus, and which Strasburger calls "Verbindungsfäden."

the same phenomena. Aside from differences in the prominence of the separate features which are to be made out from a comparison of these results, the following variations may be mentioned. The form of the nuclear structure may vary greatly in different objects, from the almost truncate cask-shape to the very pointed spindle, as, for example, in the parent spore-cells of Psilotum (Taf. VI. Figs. 86, 90), or Equisetum (Figs. 102, 105, 107); but advanced stages usually exhibit in all cases a very plump outline. The nuclear plate may be homogeneous, as though formed by the complete consolidation of its rodlike bodies (Allium, pp. 137, 138, and Taf. VI. Figs. 55, 56); the rods may be few (Taf. VI. Fig. 53), or numerous, large, and closely approximated, as in Psilotum (Taf. VI. Fig. 87). The interzonal filaments increase in number and in size, as, for example, in the parent cells of pollen in Allium (p. 138) and Tropæolum (p. 140), of spores in Equisetum (p. 149), and of macrospores in Isoëtes (p. 158); but owing to the smallness of the nucleus, they do not always become convex enough to reach the wall of the parent cell. The granular accumulation in the equator leads to the formation of a continuous structure, the cell plate (pp. 27, 111, etc.), in which is differentiated the cellulose partition of the two new cells. The halves of the cell plate, not receding from each other to any such extent as did the halves of the *Kern*platte, form the "Hautschicht" of the young cells. Inasmuch as the nuclear structure does not always swell in its equator sufficiently to meet the wall of the parent cell, this cell plate may be supplemented by a similar structure in the surrounding protoplasm (Plattenschicht im Protoplasma, pp. 28, 111, 113, etc.) continuous with it. The adjacent protoplasm may then show a fibrous differentiation (see Taf. II. Fig. 30) similar to and parallel with that of the nucleus. Thus both nucleus and cell protoplasm may operate conjointly in accomplishing the formation of the new boundary, or, as in Spirogyra, they may act simultaneously, but separately, for the accomplishment of the same object. Only a single nucleolus makes its appearance in the new nuclei of Ulothrix, while in many nuclei peculiar differentiations appear in the form of granular bands running parallel to the spindle axis (p. 27, Taf. II. Figs. 29, 30), or granules are arranged (pp. 96, 119, 138, Taf. V. Figs. 28, 37, and Taf. VI. Figs. 62, 65, 110) in the equator of the new nucleus, in a plane transverse to the axis of the spindle.

The process of division is somewhat abbreviated in the case of the formation of spore and pollen cells. The nucleus of the parent cell is divided, with the formation of a "Kernplatte," into two, and the "Zellplatte" is indicated; but before a cell wall can be formed the two new

nuclei again divide in the same manner, so that the wall of all four cells is formed almost simultaneously, although the division of the parent nucleus was by two successive steps.

Still more remarkable is the abbreviation which prevails in the formation of spores in Anthoceros, and makrospores in Isoëtes, for here the spindle filaments are formed between two masses of protoplasm, *while the parent nucleus still retains its form*, but the usual nuclear plates are not produced. Each of the protoplasmic masses (potentially, though not formally, a nucleus) again divides, without forming a nuclear plate, and in each of the four masses arises a single nucleus, not, however, until the parent nucleus, after remaining thus long, has finally disappeared. These abbreviated forms of division serve as a possible explanation of the phenomenon of free cell-formation. In the case of Picea vulgaris, for example, after dissolution of the "Keimkern" (equivalent to the nucleus of the first segmentation sphere in animals), normally *four cell nuclei arise simultaneously* in the upper end of the "egg" (p. 21). These exercise an influence on the surrounding protoplasm, which is thus made to assume a radially striate appearance about the nuclei as centres. The boundary of the four corresponding cells then makes its appearance, and what is left of the substance of the egg-cell furnishes to these four cells (or their descendants) albuminous matter. In the case of another conifer (Ginko biloba) there are as many as thirty new nuclei, which simultaneously take the place of the primary nucleus, and about these, as centres, all the protoplasmic contents are divided into a corresponding number of cells.

In the formation of free endosperm cells of Phaseolus multiflorus the nucleus and cell make their appearance *at the same time*, — not the nucleus first, as has usually been maintained, — the former in almost punctiform size, the latter as a clear, circular zone surrounding the former. Both increase in size: the zone often exhibits a radiate arrangement of protoplasmic particles about the nucleus as a centre. The nucleus remains for some time homogeneous, and then several vacuoles simultaneously make their appearance. The protoplasm of the cell becomes reticulate; the nucleus assumes an eccentric position; the protoplasm finally takes the form of a thin, granular mural layer, free from vacuoles; the cells, by their increase in size, come into mutual contact; and it is only then that a cellulose layer is to be discovered.

The radial structure of the zone surrounding the nucleus in the case of the "Ei" of Ephedra — where the free cell-formation is much like that of the last case — is particularly distinct, and the protoplasm of

each new cell exhibits a differentiation into a more compact portion immediately surrounding the nucleus, and a peripheral, less compact portion.* The nucleus differentiates a nuclear membrane and nucleoli of varying size, and the cells produce a cellulose envelope before their mutual contact.

In his "General Results and Considerations," based on animal as well as vegetable cells, Strasburger discusses first the stellate phenomena. In free cell-formation there are in operation forces which, acting from a central mass, attract most of the molecules of the surrounding protoplasm, but repel a small part of them, namely, those which compose the "Hautschicht." These are molecular forces, and the radial arrangement of the protoplasm favors the view of a polarity of the protoplasmic molecules. In the eggs of animals, at the first formation of the "Keimkern," it is surrounded with rays. It is by means of these rays that the nucleus *pushes itself* away from the peripheral Hautschicht, until, having reached the centre of the egg, it remains in equilibrium with its rays, reaching out to the Hautschicht on all sides. It may remain in an eccentric position only when the sphere of its operation is too limited to cause it to take the central position.

It seems to me unfortunate for this explanation that the influence of the nucleus, as expressed in the length of the rays, should in some cases diminish as it nears the centre of the egg. How, too, will it be possible to explain the migration of the male pronucleus when, as in Limax, there are no protoplasmic rays to push it from the periphery?

The function of the nucleus, he says, is made most obvious in cell division. It becomes homogeneous, then an opposition between two points of its surface is developed. These points mutually repel each other, and a lengthening of the nucleus is the result. Certain components of the "Kernsubstanz" are repelled from each of the two poles, and are collected simultaneously to form the nuclear plate. In proportion as the substance of the nuclear plate recedes from the poles, a striation is distinguishable behind it. These changes within the nucleus induce the radial alterations in the surrounding granular protoplasm. The changes in the form of the cell are accomplished by the influence of the nucleus, but only when the rays have reached the surface of the protoplasm (Ascidia).

Although the "Kernfäden" do not seem to have in animal cells an extensive development or well-marked function, in plant cells they

* This recalls the condition of the eggs of certain echinoderms, quite recently described by Ed. van Beneden and Selenka.

increase in number and volume, and are employed for the construction of the cell plate. Their exact signification in the formation of the "Hautschichtplatten" may not warrant his earlier (first edition) conclusions as to the identity of nuclear substance and "Hautschicht." He is now inclined to believe that there is simply an accumulation of "Hautschichtmasse" *between* the Kernfäden, especially since the Hautschichtplatte is formed in some places without the assistance of these filaments; the real signification of the latter may, perhaps, simply be to guide the substance necessary for the formation of the plate in the proper courses, and possibly to afford (mechanical?) support to the plate. From the manner in which the Hautschicht is preformed along the future plane of division in plant cells it is certain that it cannot be compared to the denser layer produced at the surface of liquids by superficial tension. He also thinks the "Einschnürungstheorie" is disproved by this observation.

Strasburger can hardly be justified in extending the latter conclusion to animal cells, especially since there is clear evidence of an infolding of peripheral substance in cases (e. g. Rana) where pigment, at first limited to the superficial portions of the cell, follows the deepening constriction.

The splitting of the cell plate ensues certainly under the influence of the two nuclei, perhaps from reasons similar to those which cause a splitting in the nuclear plate.

Although he claims for the nucleus a controlling influence in the process of cell division, he is compelled to admit in certain cases (spores of Anthoceros, macrospores of Isoëtes, etc., where the old nucleus is "pushed to one side and finally dissolved," while the function of division is assumed by an "Attractionsmasse" which is individualized near by) that the nucleus has lost its power of division. The explanation given is that *new nuclear substance* has been collected about the old nucleus, and has assumed the function of the latter.* The necessary phyllogenetic connection of this with the more typical division is at once demonstrated by the fact that the nearest relatives of these plants (indeed, the *micro*-spores of the *same* Isoëtes plant) exhibit the normal method of cell division in their spores.

I have no objection to considering, with Strasburger, this modified form of division as the result of independent adaptations (induced, perhaps, by the same causes) in each of the cases which he cites; but I cannot consider the process so fundamentally different from the typical

* "In der That sahen wir diese am Kern angesammelte Masse mit ihren Stärkeeinschlüssen sich ähnlich wie sonst die Substanz der Kerne bei der Theilung verhalten."

method as he does. Our differences of opinion result from different conceptions of the *rôle* of the nuclear substance during division, — of the nature of the "Attractionsmasse," in other words. It follows from the passage which I have just quoted, that he considers the two (afterwards four) starch-containing masses as nuclear substance. I believe, on the other hand, they are accumulations of *cell* protoplasm — the equivalents of the so-called *areas* — about centres of attraction, and that the distance of these centres from the old nucleus is only another argument tending to show what I have already suggested, that the centres of attraction may perhaps at first be quite independent of nuclear substance. I think it will be at once apparent that from this standpoint there is not so wide a gulf separating the two processes as appears from Strasburger's interpretations. It no longer becomes necessary to assume, with him, the existence of a *new* nuclear substance, to which are transferred the functions of the old nucleus, while the latter maintains a separate existence. Nor are we forced to look upon the dissolution of the old nucleus as essentially different from the metamorphosis which takes place in the typical case. Barring the assumption that the centres of attraction are nuclear substance, the metamorphosis consists essentially in the transfer of nuclear substance in two (or more) directions *toward* centres of attraction. That such a transfer of nuclear substance (from the old nucleus) also takes place in these exceptional cases must be granted, I think, from Strasburger's own words, for he says (p. 158): "Der Mutterzellkern wird aber inhaltsärmer, während seine Hautschicht dicker und granulirter erscheint (Fig. 3); endlich schwindet er vollständig. Es fällt dies sein Verschwinden mit der Zeit zusammen, in der die Zellplatten gebildet werden. . . . . *Um diese Zeit beginnt aber auch erst die Differenzirung der Zellkerne* in den vier Protoplasmamassen. Die Anlage beginnt immer seitlich von der Stärkemasse und zwar, so weit sich dies noch sicher stellen lässt, auf derjenigen Seite, welche der letzten Theilungsfläche zugekehrt ist." From this I can only conclude that the dissolution of the old nucleus is accompanied by a transfer of its substance toward the four centres of attraction near which it is employed in the formation of the four new nuclei. That these new nuclei lie on that side of their respective starch-enveloping masses which was last in union with a cognate mass, only serves to confirm one in assuming that a *part* of this nuclear substance had been transferred to the vicinity of the "Attractionsmasse" before the second division of the latter ensued; that assumption, moreover, seems in no way to conflict with the statement that the old nucleus was at this time

becoming "inhaltsärmer." Whether this transfer of substance is accomplished in the form of visible granules, in any way comparable with a nuclear plate, seems doubtful from Strasburger's description; but if his studies were confined to alcoholic preparations, it would have been very easy, even for so accomplished an observer, to have overlooked nuclear-plate stages, which are always of comparatively short duration. I do not, however, wish to express any opinion on this point, without the personal observations necessary to an independent judgment. May it not be, however, that Tschistiakoff's (**'75**) statement — that the nuclei are found much nearer the planes of division than are the clusters of starch granules to which they correspond — is based upon the observation of stages in the migration of a nuclear plate (his nucleus), which were not seen by Strasburger? The latter observer, it is true, states that the nuclei lie at first in contact with the cluster of starch granules, and subsequently move away from the latter. So far as I know, however, such a migration *away* from an "Attractionscentrum" has not been observed by others.

In Strasburger's opinion the least modified methods of cell division are such as prevail when the whole cell contents are granular protoplasm, and the nucleus is central. Here the nucleus plays an important *rôle* (through the interzonal filaments) in the formation of the cell wall. All other causes are, to a greater or less extent, modifications of this. It is shown, further, that the share which the nucleus has in this process may be gradually diminished, and for it may be substituted the activity of the mural protoplasm. The formation of the cell partition may thus, in place of being *simultaneous*, at length be brought about by the *successive* steps of an ingrowth from the mural protoplasm. The function of the nucleus in cell division has thereby suffered a reduction, and an ultimate condition is to be found in such cases as Cladophora,* where the

* P. S. — Schmitz (**'79**) has brought forward evidence to show that, contrary to the opinion held by Strasburger, the bodies which he ('**76**, p. 87) saw in Cladophora, and designated as "halbkugelige Anhäufungen körnigen Protoplasmas," are really cell nuclei. Schmitz has shown, among other things, that they behave like nuclei when treated with reagents, and has followed them during division, in which, however, he has been able to gain only unsatisfactory evidence of a filamentous differentiation. An elongation always takes place prior to division, and a diminution in the intensity with which the body stains is at this time accompanied by a gradual massing of the nuclear substance at the two poles. The nuclear division is positively not accompanied by a division of the protoplasm, so that from the uninuclear germ there results first one and then a number of *multinuclear* cells. He corroborates Strasburger in making the cell division, when it does occur, quite independent of these nuclear structures.

nucleus has become superfluous, and has consequently disappeared entirely from the development.

The substantial identity of cell division with plants and animals allows Strasburger to conclude that cell formation is induced by the same forces throughout the whole realm of organized beings. Whether this identity justifies the conclusion that animal cells and plant cells are homologous, — that consequently animals and plants have a common origin, — is much less certain. It is not possible, *a priori*, to positively deny that the successive events of cell division are an *immediate* mechanical necessity. The latter cannot, for the present at least, be demonstrated; but its possibility once granted, it is clear this agreement in the succession of events no longer serves as evidence of a direct (genetic) relationship between the objects under consideration.

Other processes of cell formation — freie Zellbildung, Vielzellbildung, Vollzellbildung (or, better, Einzellbildung) — are to be considered as abbreviations of cell division. Successive stages in this process of abbreviation are demonstrable. For example, in the egg of the Abietineæ the four cells which are formed after the dissolution of the old nucleus about four newly made free nuclei are arranged just as though they had been formed by the repeated division of an apical cell. This presents a case less removed from normal division than is that where (Ephedra) the resulting cells no longer retain any definite topographical relationship. The spores of the Ascomycetæ are produced by free cell-formation, namely, a dissolution of the nucleus of the mother cell and the *simultaneous* appearance of as many secondary nuclei as there are to be spores; but in some cases it has been shown that the necessary number of nuclei arise by *successive* dichotomous division. The extremest modifications are such as occur when the old nucleus is not dissolved before the beginning of the free cell-formation, but is pushed to one side and remains unemployed, while new cells arise out of a part of the protoplasm of the mother cell, as is exemplified in the formation of the "Keimblaschen" and their "Gegenfüsslerinnen" with metasperms.

A somewhat similar case (Isoëtes) I have considered above, and am not entirely satisfied that this last one may not also be found on closer study to diverge less from the normal method of cell division than we are warranted in concluding from Hofmeister's investigations.

Animals afford less opportunity for the study of "free cell-formation" than plants, and it may appear hazardous to venture any suggestions as to a possible point of comparison. There is, however, in the embryology of some animals a method of cell production which appears to be closely

related to this process of free cell-formation, — which is to a certain extent intermediate between cell division and the latter. I refer to the formation of nuclei (by division) in homogeneous masses of protoplasm which do not at once (perhaps never) respond to the nuclear division by a division of their substance, — e. g. segmentation in Eupagurus Prideauxii (P. Mayer, '77). Such potential cells (autoplasts, Lankester; entoplasts, Whitman,) differ, as regards their origin, from the free-formed cells in that their nuclei demonstrably arise by *division*. It seems to me not impossible that the free cell-formation of botanists may ultimately be found to be much more restricted than at present believed; that it may be possible, namely, to demonstrate the existence of stages of division in the nucleus which have hitherto been overlooked or mistaken for its dissolution. I strongly suspect that such is at least the case with Strasburger's observations on the cell division of Isoëtes. The exceptional cases with Ascomycetæ point in the same direction. If such a restriction of free cell-formation should be realized in plants, the differences in the secondary modifications of cell division in plants and animals would not be so divergent as they at present appear.

Strasburger takes occasion, in considering Auerbach's views of the nature of the nucleus, to expand his own ideas. He practically distinguishes three sorts of "Kernsubstanz": one is an active kind, which is collected at the "poles," and is thereby divided into two antagonistic portions; another is repelled by these poles, and collects as a median nuclear plate; and a third kind, not repelled by the substance of the poles serves, in the form of filaments, to join the latter with the median plate. The maximum removal of the poles from the nuclear plate seems to be soon attained; but as the poles continue to repel each other, it results that the nuclear plate is thereby divided into halves. He concludes that the nuclear plate plays a passive *rôle*, since a median portion of the same is drawn out in the form of fine [interzonal] filaments.

I do not fully agree with these conclusions. Strasburger himself grants that the "nuclear poles" are "in stofflicher Beziehung von der übrigen Kernmasse verschieden," in that they are more highly refractive. It is therefore an assumption when he says the "poles" consist of nuclear substance. It is unsatisfatory to assume that the substance of the nuclear plate acquires its equatorial position in virtue of a repulsive influence exercised on it by the "poles," since it leaves unexplained how it is that the same substance subsequently *approaches* these "poles." The nuclear fibres are probably not *exclusively* nuclear substance, since it is demonstrable that they in some cases arise outside the nucleus, and it

is perhaps even more questionable if any part of the interzonal filaments is nuclear substance.

Other points of his descriptions and conclusions agree much better with my own observations. As regards the formation of the new nuclei in the segmentation spheres of animal eggs, he gives the following in many particulars excellent summary, parts of which I take the liberty to italicize: "Im Kern folgt aber dem geschilderten Zustand derjenige seiner definitiven Ausbildung, die mir in dem Gange, welche sie in sich furchenden thierischen Eiern einschlägt, besonders instructiv schien. Da beginnt nähmlich von der früheren Segmentseite an die homogene Kernsubstanz sich in dichtere und minder dichte Bestandtheile zu scheiden; die dichteren bilden das Licht stärker brechenden Kernkörperchen, die minder dichten schwächer das Licht brechenden, die Grundmasse des Zellkerns, in der die Kernkörperchen schweben. Um diese Zeit ist der Kernpol noch erkennbar und weisen die umgebenden Strahlen noch auf denselben hin, er scheint sich also nicht [direct] an der Bildung der Kernkörperchen und der Grundmasse des Kerns zu betheiligen, auch ist er von der so differenzirten Stelle durch den homogenen Theil des Kerns getrennt. Erst wenn die beschriebene Differenzirung im ganzen Zellkerne vollendet ist, beginnt auch die Substanz des Kernpols sich zu vertheilen und gleichzeitig schwindet das homogene, ihn umgebende Zellprotoplasma und die von letzerem ausgehenden Strahlen. *So lange der Kernpol und das ihn umgebende Zellplasma sowie die Zellstrahlen nicht ganz geschwunden, behauptet er auch seine Stellung und kann der ganze Zellkern noch nicht in seine Stelle rücken.* Die Polsubstanz scheint sich in der Grundsubstanz des Zellkerns zu vertheilen, ohne besondere Formelemente desselben zu bilden. Freilich ist es nicht leicht Letzteres festzustellen, doch möchte ich fast darauf schliessen, erstens aus dem schon erwähnten Umstande, dass die Differenzirung der Kernsubstanz in Grundmasse und Kernkörperchen schon vor sich geht, wenn die Polsubstanz noch als solche unterscheidbar ist; zweitens aus der wiederholt von mir bei Unio und ein Mal auch bei Phallusia beobachteten Erscheinung, *dass die Ansammlung der activen Kernmasse an den Polen ausnahmsweise* [?] *beginnen kann vor Auflösung der Kernkörperchen, beziehungsweise der Kernhülle, bevor also die Substanz des Kerns homogen geworden ist.*"

If he were to admit that the two centres of attraction are not poles of the nucleus in all cases, and were to grant that the "Kernsubstanz" of the poles is not of necessity *nuclear* substance, then our views would not differ widely. I cannot assent, however, to the relation which the new nucleus is made to hold to the centre of attraction at the early stages

of its formation. I see it *in shape* substantially as Strasburger has figured it (*loc. cit.*, Taf. VII. Fig. 21, and Taf. VIII. Figs. 9, 12), — a pear-shaped body with its blunter end directed toward the plane of segmentation (Limax, Fig. 80^{a}), but in *position* it is quite different. The more pointed end does not correspond to the centre of attraction ("Pol") as he represents it, but is removed from the "Pol" half the long diameter of the nucleus at a time when several nucleoli have made their appearance. This distance makes the more certain the conclusion of Strasburger, that the "Pol" furnishes no "Formelemente" to the growing nucleus, and has suggested the possibility that this central portion of the "area" might not be nuclear substance, and that it might not enter into the composition of the new nucleus, but become finally diffused in the protoplasm of the yolk.

The differentiation of the nucleus into more and less dense portions is not so clearly localized as in Strasburger's Fig. 21, Taf. VII., although the nucleoli are here (Fig. 80^{a}) more abundant at the blunt end. The peculiar *form* I would explain as brought about by the continued attraction of the so-called Polsubstanz upon the nucleus combined with the increasing opposition offered to its actual progression by the increasing density of the protoplasm nearer the centre of the "area." * The subsequent assumption of a more nearly spherical form would then be but the natural result of a gradual diminution of the attractive force, further evidence of which is seen in the gradually fading rays of the protoplasmic aster. Strasburger is "convinced that the form of the cell nucleus is to be taken as the expression of the forces operating in its interior." With that conception the peculiar pear-shaped form now under consideration could not easily be otherwise explained than as I have suggested in the foot-note, for the "Polsubstanz" most certainly at this time forms no part of the *interior* of the nucleus. A corresponding relationship of "centre of attraction" and nuclear bodies is shown for the pronuclei of Limax in Fig. 68. This seems to me a confirmation of the view which makes the peculiar form due to the attractive influence of substance which lies outside both the pronuclei, and which also induces a stellar figure in the surrounding protoplasm.

The most serious obstacle to the view which I have suggested is found in the formation of free nuclei, where, to make use of Strasburger's

* In view, however, of the automatic form-changes of which the nucleus is certainly at times capable, it may not be erroneous to look upon this pointed extremity as a sort of pseudopodal prolongation, having an important function in the nutrition of the nucleus from the substance of the "area."

words, "the active *Kernstoff* is uniformly distributed in the remaining *Kernsubstanz*," as far at least as the optical evidence goes. While this does not necessarily preclude the notion I have maintained, it certainly gives a great appearance of accuracy to the view which recognizes in this active (attractive) substance an essential part of the nucleus. Strasburger, however, is compelled still to recognize a separate active "Kernstoff," without disclosing to us any of its properties save those which are manifest by the radial phenomena. It therefore seems to me equally justifiable to apply to this *active* substance any other name than *nuclear* substance. If one may call it nuclear substance because it is here surrounded by or distributed through nuclear substance, then with equal propriety it may be called in nuclear division a protoplasmic (vitelline) substance.

The importance of this substance which forms the centre of the aster ("Kernsubstanz") is thus formally announced by the author: "Meine ganze Auffassung gipfelt in der zum Theil in dieser Auflage erst scharf formulirten Behauptung, dass *von der activen Substanz an den Kernpolen die ganze Structur der Kerne und die Kerntheilung, die Structur des umgebenden Zellplasma und dann die Zelltheilung bestimmt wird.*"

In a subsequent paper Strasburger (**'77**, p. 518) calls attention to a case (Nothoscordum) in which the spindle fibres are exceptionally delicate, and the nuclear plate is represented by rodlike elements arranged in part around (outside) the equator. From this he concludes that the elements of the nuclear plate are not simple swellings of the fibres, and that in this particular case their repulsion from the poles was so forcible as to result in their elimination from the spindle.*

Treub (**'79**) has contributed interesting observations on the *rôle* of the nucleus in the division of plant cells. His observations were made mostly on the phanerogams, and especial attention was given to following the steps of nuclear changes in living cells examined in a comparatively indifferent fluid. The time which intervenes between the different stages figured is given. These observations are supplemented by studies of alcoholic preparations stained in picrocarmine.

The formation of the nuclear plate is accomplished (e. g. in the "ovules" of Epipactis) without the intervention of a homogeneous condition of the nucleus. The latter, at first finely granular and containing a single nucleolus, at length contains a limited number of coarse, distinct, irregularly disposed granules. After the lapse of some time these gradually accumulate at the middle (equator) of the nucleus to form di-

* See also p. 350.

rectly the nuclear plate, in which they lose their individuality. This migration and confluence of the coarse granules is accompanied (always?) by a marked contraction of the nucleus.

In the division of the nuclear plate and the separation of its halves, there appear first a narrow dark line through the middle of the band (the disk seen edgewise), and then several detached, lenticular clear spaces, which at length become confluent and thus effect a complete separation of the halves. The latter move apart, rapidly at first, afterward with a gradually retarded motion; they become thicker, but less definitely outlined, the farther they move away from each other. They remain united more or less by irregularly placed bands of threads, which for an instant may form a bundle of parallel striations. Usually the nuclear plates remain parallel, but in one case (Fig. 7) it was observed that the central portions of the disks became widely separated while their peripheries still remained close together.*

During the latter part of the migration of the halves of the nuclear plate, the old nucleus (its outline is no longer sharply distinguishable from the enveloping protoplasm) undergoes a change of form and size. It elongates, then it becomes broader till it sometimes touches the wall of the cell; but later its diameter undergoes a considerable reduction. The nucleus finally becomes cylindrical, and at the same time the halves of the nuclear plate become gradually rounded, and may now be called secondary nuclei. Still later, minute granules in active motion are seen to make their way toward the middle of the space between these two secondary nuclei. They arrange themselves in a transverse layer, — the beginning of the cell plate. Whence they arise is uncertain, but since they move in all directions it is hardly possible that they glide along invisible filaments stretched between the two secondary nuclei.

In Crinum asiaticum Treub has seen the nuclear mass differentiated into rods (Fig. 27),† instead of granules, from which the nuclear plate is formed. The homogeneous condition of the nucleus before the formation of the nuclear plate is far from being general.

Treub disagrees with Strasburger as to the methods in which the cell

* Were it not that Treub's sketches presented in this case stages quickly succeeding each other (Figs. 7^b to 7^h = 20 min.), I should be inclined to think that his Fig. 7^c was the representation of a single annular nuclear disk seen somewhat obliquely. The signs of division shown in Fig. 7^b seem to preclude that view. I am induced by these observations to again call attention to Fig. 24 of Bobretzky's ('76) paper, and to admit that possibly a similar condition to that seen by Treub is the basis of this figure, which I have endeavored to explain in quite another manner.

† Compare Strasburger '77, Taf. XXXIII. Figs. 55–58.

plate and cellulose membrane are formed. He says (p. 28): "(1.) The cell plate, formed in the 'cask' between the two young nuclei, grows at its edges until it touches the walls of the cell on all sides. (2.) Never have I seen the cell plate formed in the cask completed by a ring emanating from the cell wall; neither have I ever seen an annular membrane of cellulose rise up to encounter the cell plate." Thus the *rôle* of the nucleus is much more important than Strasburger admits. It is by the direct intervention of the young nuclei that the whole cell plate, and consequently the whole of the cellulose membrane, is formed.

From a consideration of all his own observations he concludes that the filaments and striations in or between the nuclei (nuclear fibres and interzonal filaments) are of secondary importance, at least in the cell division of the higher plants.

2. *Maturation.*

The fate of the germinative vesicle has been a matter of ardent discussion since 1850, yet it is only within a very recent period that sufficiently varied methods of investigation have been employed to make the attainment of a final decision probable. But increased facilities of study have not led to unanimity of opinion among those who have carefully followed the changes which overtake this structure.

It is now very generally conceded that the germinative vesicle, like the nucleus of an ordinary cell,* suffers a remarkable metamorphosis. By some observers this metamorphosis is claimed to be only a rather fundamental rearrangement of the constituents of the vesicle, without their general dispersion; by others, that it is so radical as really to involve a total dissolution and a distribution of the nuclear matter. To this latter conception the term *metamorphosis* can be applied only in its broadest sense; on the other hand, the first conception is not held to entirely preclude either the loss of old, or the acquisition of new matter in the process of transformation.

* A general discussion of the morphological value of the germinative vesicle cannot be attempted here, especially since the origin and growth of the egg, which has formed no part of my studies, must of necessity constitute an important portion of the evidence to be considered in such a discussion. I may state, however, in this connection, that quite recently a view, which at one time obtained very general acceptance, has been revived by Brandt, Villot, and others. They claim that the germinative vesicle is entitled to rank as a *cell* rather than as a *nucleus.*

Without attempting to refute at length their position, I wish to emphasize the fact that the germinative vesicle sustains the same relation to the first maturation spindle that an ordinary *nucleus* does to the spindle which takes its place.

These two views more or less closely reproduce the opposing ideas held by the earlier embryologists. An extended review of those earlier opinions is rendered unnecessary by the labors of recent investigators, especially Oellacher, Flemming, Fol, O. Hertwig, Bütschli, Ed. van Beneden, and Whitman. The different views group themselves naturally under one or the other of three heads, according as it was claimed that the germinative vesicle disappears, or persists and undergoes division, or, finally, that only the germinative dot remains while the vesicle suffers dissolution.

Although it has often occurred that the same author has arrived at opposite conclusions in the study of different animals, it does not follow that differences of opinion can in general be referred to the actual existence of differences from one species to another; on the contrary, it has been possible for the study of the same species to lead to the most divergent conclusions.*

But if the germinative vesicle does not persist and undergo division to form the nuclei of the first pair of segmentation spheres, what becomes of its substance? This question has had various answers. Purkinje (**'30**, p. 15), the discoverer of the vesicle, entertained the opinion that, inasmuch as it was not to be found in (hen's) eggs taken from the oviduct, it became ruptured by the contractions of that organ, and that its contents, a "lympha generatrix," became mingled with the germ. It was owing to this supposed germinative influence of the contents that the vesicle was thus named. Von Baer (**'27**), who was the first to show that a migration of the vesicle takes place in the hen's egg from the centre to

* In the case of the rabbit, for example, Bischoff (**'42**, pp. 38, 39, 75–77, 141) concludes that the germinative vesicle, which exists as a protective envelope to the germinative dot up to the time of impregnation, disappears, and that the dot under the influence of the male element undergoes division, the two parts becoming the centres about which the yolk is grouped to form the first pair of segmentation spheres. Ed. van Beneden (**'70**, pp. 178, 179), on the contrary, holds that the disappearance of the germinative vesicle is apparent rather than real, just as it is an untenable position to maintain that the nuclei of segmentation spheres, because they become pale, homogeneous, and transparent, disappear and are replaced by new nuclei. Also at p. 244 (*loc. cit.*) he says: "En résumé je considère non comme démontré, mais comme très-probable que la vésicule germinative se divise au lieu disparaître, et que ses portions deviennent les noyaux des deux premiers globes vitellins."

Bischoff (**'45**, pp. 22, 42, **'52**, pp. 20, 21) subsequently modified his views in so far as to consider the persistence even of the germinative dot as problematic, and Van Beneden (**'76**[a], pp. 39, 40, and **'76**[b], p. 154) now goes so far as to assert that his "researches on the ovum of the rabbit have proved . . . . that no morphological part of the germinal vesicle is found in the yolk at the moment of fecundation."

the periphery, and probably also in the case of other animals, held nearly the same opinion concerning its fate and function.* Its disappearance, however, was not due to the contraction of the oviduct, but resulted from the maturation of the egg. Van Bambeke (**'76**, pp. 116, 117) has recently described for batrachians a "claviform figure," which indicates the course pursued by certain parts of the germinative vesicle at the time of their *expulsion* from the egg. The dilatation at the internal end of the club-shaped figure corresponds to the place occupied by the germinative vesicle at the moment of its disappearance, after which event one finds at the superior pole of the egg traces of the expelled portions. He is not able to say what parts are expelled, and what remain in the vitellus.

The dissolution and elimination of the vesicle has been very generally believed in by those who have followed the development of vertebrates other than mammals, and in a modified form has recently had an able advocate in Oellacher (**'72**). This author has endeavored to harmonize the phenomena observed in mammals with his own careful observations on the trout egg, by considering the so-called polar globules of the former the equivalent of the *contents* of the germinative vesicle in the trout. The latter assumes, as he has discovered, the shape of one or two spheroidal masses at the time when the membrane of the germinative vesicle is everted and spread on the surface of the germ. He concludes (*loc. cit.*, pp. 24, 25) that the germinative vesicle in all vertebrates' eggs, as they approach maturity, migrates to the surface and is ejected from the germ; that in no *vertebrate* is there a genetic connection between the vesicle and the nuclei of the first segmentation spheres; and that the same is possibly true of all animals, as observations on the eggs of mollusks would help to prove.

In the opinion of these and many other authors the vesicle (or parts of it) is eliminated in an amorphous condition, or promptly becomes such and then vanishes. Not widely different from this view is the opinion which early gained credence with students of invertebrate embryology, connecting the germinative vesicle with the formation of discrete spheroidal bodies, first observed in the case of mollusks,† which are detached from the vitellus as "polar globules."

* "Post fecundationem verum blastoderma eo loco evolvitur, quo vesiculæ humor effusus est." (*Loc. cit.*, p. 29.)

† So far as I know, such a structure was first figured by Carus (**'24**, Taf. IV. *A. a*). However, he gives no very intelligible description of it in the text. Several years later, Dumortier (**'37**, pp. 10, 11, 15, and Pl. I.) saw such bodies, two in number,

But among those who have insisted on the derivation of the polar globules from the germinative vesicle, there has been considerable diversity of opinion. As we have just seen, Dumortier held the globules to be the vesicle itself. Van Beneden and Windischmann inclined, as previously stated (p. 236), to the same opinion.

Lovén (**'48**, pp. 535 – 539), on the other hand, expressed, with some reserve, the opinion, that in lamellibranchs it was the germinative *dot* which, after rupture of the vesicle, became expelled. Leydig (**'49**, p. 125) conjectured the same to be true for Piscicola, and Bischoff (**'42**, pp. 54, 146) held to the idea that the globules observed by him in the rabbit were at least derived from the germinative dot.

More recently Flemming (**'74**, pp. 278, 279) has maintained that it is neither vesicle nor dot, but a product of the metamorphosis of both,

and believed them to be the Purkinjean vesicle. They were observed in the case of Aplysia by P. J. van Beneden (**'40**, pp. 242, 243, 245) who called them "vésicules blanches," and in the same paper mentions their occurrence in Limax, although the first account of their formation in Limax was that given by Van Beneden and Windischmann (**'41**, pp. 20, 21) in the year following.

Fr. Müller (**'48**, p. 3) attributed to these bodies an importance in determining the position of the planes of segmentation, and hence gave them the current German name "Richtungsbläschen."

Robin (**'62**[d], p. 150), also recognizing their constant relation to the early segmentation planes, calls them "globules polaire," but believes that they are derived, not from the germinative vesicle or germinative dot, but from an accumulation of clear yolk at one pole of the egg, and that granular portions of the yolk may secondarily make their way into the globules while the latter are in process of formation. Several observers have alluded to the name polar globules as though it were first used by (the elder ?) Van Beneden. I have not succeeded in finding the use of that expression in the earlier writings of P. J. van Beneden, and am therefore of the opinion that Robin was the first to make use of this term.

P. S. — Fol (**'79**, p. 146 or p. 58 of separate) casts doubt on the nature of the observations made by Carus and Dumortier. He says they are currently, in his opinion wrongly, believed to have discovered the polar globules. "It has been impossible for me," he adds, "to find in the works of these authors the description of corpuscles which are referable with probability to the polar globules."

I have likewise been unable to find any description in Carus referable to polar globules, unless the allusion (*loc. cit.*, p. 53) to "hellere durchscheinende Stellen (*a. b.*)," which one distinguishes "an zwei polar entgegengesetzen Punkten" may refer to phenomena which in some cases accompany the formation of polar globules; namely, the accumulation of clear protoplasm at the vegetative as well as animal pole of the egg. The protuberance outlined in the figure (IV. *A. a.*) above referred to represents too closely a nearly completed polar globule to permit assigning any other signification to it, although it may count for little, that, among many projections from the surface of the embryo Carus has chanced to figure, without understanding its meaning, one of those structures over which there has been so much discussion. — With

which is expelled in the case of the acephalous mollusks. His opinion, although subsequently substantiated, was not based on a satisfactory knowledge of the exact nature and sequence of events in the supposed metamorphosis.

What relation, if any, exists between the disappearance of the germinative vesicle and fecundation? or, granting the derivation of the polar globules from the germinative vesicle, what is the relation between fecundation and the formation of polar globules?

Many excellent observers have raised these questions without being able to arrive at a definite conclusion. Thus Lovén (**'48**, p. 535) is uncertain, in the case of Modiolaria, whether the migration of the vesicle to the surface of the yolk and the dissolution of its membrane are processes which pertain to the life of the egg or to the development engendered by

regard to Dumortier (**'37**, pp. 10, 11, 15, Pl. I.) the case seems much clearer. Six hours after the egg is deposited "on remarque sur le côté un hile muqueux et diaphane (Fig. 1, *K. a*) qui est la vésicule de Purkinje." Notwithstanding the erroneous notion of the ultimate fate of this "hile muqueux," I think there is not only a probability, but almost a certainty, that it is the polar globule. The recent deposit of the egg is in itself, perhaps, enough to make this interpretation probable, especially in connection with the statement that the egg is at this time always *totally round* and *opaque*; but there is additional evidence in the further description given by Dumortier. In his account of the egg on the second day he says: "Le hile de son côté s'est prolongé et paraît formé de deux globules diaphanes, qui ne tardent pas à se séparer et à se détacher l'un de l'autre (Fig. 2, *C. a. b*). . . . . Toutefois le hile disparaissant complétement le 4ᵉ jour pour ne reparaître que le 8ᵉ jour." So far as I know, there is only one phenomenon beside the formation of the polar globules to which this description could possibly apply; viz. the elimination of drops of an entirely transparent fluid previously accumulated between the segmentation spheres. There is little probability that these descriptions are applicable to the latter process. In the first stage the complete rotundity and the opacity of the yolk make it improbable that the first segmentation had been completed; for (although the first two segmentation spheres become mutually flattened so as to give the whole *approximately* the spherical form again) the accumulation of the liquid would have led the observer to qualify his assertion that the yolk was opaque; but if the first segmentation had *not* taken place, then the hilum could not have been due to the accumulated liquid, — at least, I am not aware that any one has observed such accumulations previous to the mutual flattening which follows the first cleavage. In the second stage the description and figures (Figs. 2 *B*, 2 *C*, 3 *B*) of *two* transparent globules, which do not disappear till the fourth day, can certainly have nothing whatever to do with the elimination of drops of clear fluid, which Dumortier himself saw and subsequently very well described (see p. 15, and description of Fig. 8 *B*, p. 44) as "une goutelette de liquide qui s'étendit bientôt dans l'albumen comme une goutte de lait qui tombe dans l'eau." Since, then, these descriptions do not relate to this elimination of liquid, I think it will be difficult, if indeed possible, to find any better interpretation than the one which they have for many years enjoyed.

fecundation. De Quatrefages ('**48**) indicates very clearly that the germinative vesicle disappears in *un*fecundated eggs (p. 171) of Hermella, but also that the "clear space" which occupies the yolk previously to the elimination of the "globule transparent" (polar globule) in eggs *already fertilized* is no longer visible after the formation of that globule. He is uncertain whether the disappearance of the germinative vesicle is accomplished in the same manner in both cases. Although he thinks Bischoff speaks too positively in saying that there is no definite relation between the time of the vesicle's dissolution and either the escape of the egg from the ovary or *the act of fecundation* (pp. 173, 174), still he ultimately (p. 181) leaves this question in an undecided condition.

Other observers have answered with sufficient positiveness, but their conclusions have differed widely. While in the opinion of many the disappearance of the vesicle followed fecundation as its immediate result, others have as positively denied such a causal relation.

Already in 1827 Von Baer maintained that the migration and disappearance of the germinative vesicle was a phenomenon of the ripening of the egg,* an opinion which he has since taken occasion to reiterate. (See Von Baer '**35**, pp. 4, 9, and '**37**, pp. 28, 157, 297.) The same conclusions have been reached by many other observers upon all classes of animals.

Reichert, on the contrary ('**46**, pp. 199, 205), evidently looks upon the disappearance of the vesicle as the first result of impregnation in the case of Strongylus, and Krohn ('**49**, p. 5, foot-note) dissents from Derbès's opinion ('**47**, p. 83), when the latter makes the germinative vesicle (sphere moyenne) in Echinus disappear *before* fecundation, and ascribes the supposed error to want of careful study. Krohn himself observes the absence of the vesicle and dot half an hour *after* fecundation (p. 7).

In 1853 Leuckart ('**53**, pp. 921, 922) thus summarized earlier opinions: "As a rule the disappearance of the germinative vesicle is considered as the immediate result of fecundation." But, on the strength of the evidence before him, he did not hesitate to draw this conclusion: "If we put all these facts together, then it really can hardly remain longer doubtful, that the disappearance of the germinative vesicle characterizes a process which belongs more to the formative history of the egg than to the history of the development of the subsequent embryo." There are, nevertheless, many who have still insisted on the essential impor-

* "*Vesiculam Purkinji* partem ovi efficacem esse credo, qua facultas feminina vim exerceat, ut facultas masculina semini inest virili. Vesiculæ igitur protusio et dissolutio ab ovi maturitate et forsan irritatione penderent." (Von Baer '**27**, p. 29.)

tance of fecundation for effecting the changes referred to. Among these may be mentioned A. Müller ('**64**), Haeckel ('**74**, pp. 141–143), and Bütschli ('**76**, pp. 388, 389), who so recently as 1876 makes this sweeping assertion: "Diese Frage nach dem Austritt des Keimbläschens vor oder nach der Befruchtung ist jedenfalls der Mühe werth, näher erörtert zu werden, denn es stimmen alle vertrauenswürdigen Untersuchungen an wirbellosen Thieren darin überein, dass die Ausstossung erst nach der Befruchtung stattfindet." But with more recent discoveries this question seems to have come nearer a final solution in agreement with Leuckart's conclusion.

The reviews which follow cover only comparatively recent observations, — principally such as have a bearing on the metamorphosis of the germinative vesicle and the formation of the polar globules.

According to Ratzel und Warschawsky ('**69**, pp. 548, 549, Taf. XLI. Figs. 1, 2) the development of the fecundated egg of Lumbricus agricola begins with a change in the germinative vesicle, which surrenders its sharp outline and becomes a "stark lichtbrechender, unregelmässig strahliger Fleck etwas excentrisch gelegen," and with the formation of a narrow, clear streak in the middle of the egg underneath the altered vesicle. This streak subsequently increases in length. Later, both structures disappear. That which entitles these observations to a notice in this connection is the clear streak which remained to the observers of doubtful signification. In my opinion they saw in this streak, however incompletely, the maturation spindle in process of formation out of the germinative vesicle. I believe this view is supported by the observed *lengthening* of the streak, which has so often been seen to take place with the spindle figure. Were it not for the absence of polar globules, which are figured only in later stages (*loc. cit.*, Figs. 3–5), I should consider this as probably the first cleavage spindle, since I have seen the same relative position of the pronuclei to the spindle in the case of Limax. The failure to connect this figure, or the germinative vesicle in any way, with the production of polar globules, cannot be surprising, as the authors do not seem to have bestowed any attention on the production of what they, with Rathke, held to be only meaningless, squeezed-out portions of the yolk.

Those eggs which do not develop retain the germinative vesicle, but its contour becomes less distinct.

Ratzel ('**69**, p. 565, Taf. XLII. Fig. 5) saw more than any of his predecessors. He says: In the eggs of Tubifex, which are mature and ready for deposit, the germinative vesicle surrenders its spherical form

and sharp membranous limitation from the yolk, which it hitherto possessed, and becomes an elongated body. Its coherence and elasticity make it possible by gentle pressure to remove it from the yolk without changing its form or size. In regard to its composition, it presents a peculiar appearance. Its middle portion is, in comparison with its poles, swollen, and exhibits a meridional striation, which results from the presence of a membranous envelope. Inasmuch as the remaining [polar] portions of this modified germinative vesicle present no trace of a membrane, and inasmuch as the median swollen portion agrees very well in size with the [unaltered] germinative vesicle, the whole structure may be considered as having arisen by the accumulation of protoplasm at two opposite poles of the vesicle. The germinative spot had already disappeared.

The expression "meridionale Streifung" might lead one to suppose that an equivalent of the spindle fibres had been observed, were it not that the "Streifung" shown in the figure (Fig. 5) has a direction at right angles to the axis of the supposed spindle figure. Although no stellar structures are indicated, I think one must conclude that the figure represents an early stage in the formation of the amphiaster, whose rays may easily have escaped detection. Whether the centres of the hypothetical asters are to be imagined as occupying the *centre* of the clear areas, or whether they correspond to the extreme points of the figure, cannot be definitely concluded. That the astral centres *may* lie at considerable distance from the periphery of the nucleus, has been sufficiently insisted upon already in the detailed account of Limax. The folding of the membrane, to which is due the striate appearance, entirely precludes the interpretation of the central mass as an oblique view of the nuclear disk. Polar globules are not mentioned.

Afterwards Ratzel (**'69**[b], p. 282) endeavors to correct the observations made by himself and Warschawsky on Lumbricus, as far as regards the disappearance of the germinative vesicle, which he now maintains gives rise by its *division* to a number of clear spots.

As I have briefly stated before, Oellacher (**'72** and **'72**[b], pp. 406–410) has described the process by which the germinative vesicle is eliminated from the egg in the case of the trout, and also in that of the hen. (See also Oellacher **'70**.) By the contractions of the protoplasm of the germ, the vesicle is forced to the free surface, where it becomes ruptured, and, in the case of the trout, its thick wall is spread out on the surface of the germ as a flat, round veil. The contents of the vesicle thus set free appear in the form of one or two finely granular spherules on the

outer surface of the germ. What becomes of them is not known with certainty, though numerous small granules scattered between the segmentation spheres at a later stage are thought to have possibly resulted from them.

A nucleus was only once seen in the germ before segmentation, and then not carefully studied. He thinks it certainly had no connection with the germinative vesicle, the latter having been already eliminated. Much of the value of Oellacher's work is due to his employing the section method with the objects studied. The streaked appearance of the germ as portrayed for two sections (**'72**[b], Figs. 27, 28) is of interest as suggesting the persistence of nuclear matter in the germ, and as possibly showing a gyratory tendency in its substance (Fig. 28) not unlike that seen in Limax.

Kleinenberg (**'72**, pp. 42, 46, 47), in his well-known paper on Hydra, describes to some extent the regressive metamorphosis of the germinative vesicle, which occurs long before fecundation. The germinative dot first becomes disintegrated and dissolved. The vesicle is forced to the external pole of the egg, where it undergoes a fatty degeneration and finally disappears altogether. A contraction of the vitellus takes place soon after the disappearance of the germinative vesicle, and is uniformly accompanied by the elimination of a few particles of the egg substance, which the author identifies with the polar globules * of other animals. No genetic connection between vesicle and polar globules was discovered.

Ray Lankester (**'73**, p. 85) affirms for Aplysia, that "the germinal vesicle escapes previously to yolk cleavage as the 'Richtungsbläschen.'"

Notwithstanding his valuable contribution to an intimate knowledge of the nuclear changes during cell division, I think we are justified in presuming that Schneider (**'73** p. 113) has overlooked some of the phenomena accompanying the earliest changes of the egg. Bütschli (**'76**, p. 399) with reason questions the propriety of his calling the nucleus of a fecundated egg the germinative vesicle. In this particular case it would seem as though the egg represented by Schneider in Fig. 5. *a*, Taf. V. embraced still the germinative vesicle, containing, as the

* The criticism of Bütschli (**'76**, p. 384), that the existence of a "Pseudozelle (Dotterkern)" in these particles makes it more than probable that they have nothing to do with polar globules, would now be without weight, for it was made at a time when the cell nature of these structures was not understood. The probability that these are polar globules receives also a certain amount of confirmation in the recent studies of Korotneff (**'76**) on Lucernaria, a review of which is given farther on.

latter does, a single nucleolus with a minute fluid-filled space. The most natural explanation of the case would then be, that the spermatozoa observed and figured within the yolk had not led, up to the time of observation, to a real fecundation;* that the description of the metamorphosis of the "germinative vesicle," as elsewhere (p. 278) given *in extenso*, relates to that vesicle rather than any other nuclear structure; but that the author overlooked the formation of polar globules, and *assumed* that the condition of every unsegmented egg found to present some stage of the rosette figure must have resulted *immediately* from the metamorphosis of the germinative vesicle, rather than through the intervention of any other nuclear body.

The changes of the "germinative vesicle" of the fecundated egg described by Fol (**'73**) for Geryonia have been given elsewhere (p. 279), since they relate to the nucleus of the first cleavage-sphere, not to the germinative vesicle. The metamorphosis of the latter escaped him. The "Faltenstern" of the egg membrane is supposed by the author to indicate the spot where fecundation takes place; but I think it is more likely that it is connected with the formation of polar globules, of which the author usually saw one.† What that intimate relation is which the polar globule in other cœlenterates sustains to the act of fecundation, the author does not say. One is inclined to believe that he looked for an orifice ‡ *out* of which the polar globule had come, and through which the spermatozoa had penetrated *into* the egg.

Balbiani (**'73**, p. 84) denies that there exists in spiders any connection between the nuclei of the blastoderm cells and the Purkinjean vesicle. He adds nothing to our knowledge of the metamorphosis of this structure.

Bütschli (**'73**[a], p. 101, Taf. XXVI. Fig. 61[d], I.–IV.) unquestionably saw and figured what is now known as the female pronucleus, but was unable to give positive information concerning its origin. It makes its appearance as a clear vesicle, at the pole of the egg which is directed toward the vagina, some time after the germinative vesicle has ceased to be visible. Whether the latter is ejected, or simply has become

* The observation of unaltered spermatozoa within the yolk gives reason to suspect that the egg here figured was not capable of normal development.

† "Vermuthlich entspricht der Faltenstern der Stelle wo die Befruchtung stattfand. Hier befindet sich in der Hülle fast constant ein Korn oder Richtungskörperchen von 15–20 μ Grösse. Ein ähnliches Körperchen, welches mit dem Befruchtungsacte in näherer Beziehung zu stehen scheint, habe ich auch bei anderen Coelenteraten beobachtet." (p. 475.)

‡ "Eine Oeffnung ist beim befructeten Ei hier [i. e. at the Faltenstern] nicht zu entdecken."

obscured, Bütschli is unable to say, but evidently inclines to the latter opinion.

Schenk ('**73**, p. 369, Fig. 4) points out the existence of a small cavity in the fecundated eggs of Raja quadrimaculata, which has a triangular outline and opens by a narrow orifice at the surface of the formative yolk. It occupies the place of the germinative vesicle.

Villot ('**74**, pp. 201, 202) informs us that the germinative vesicle has apparently disappeared in the eggs of Gordius at the time of deposit, but the subsequent contractions of the vitellus bring into view a nuclear structure, which he insists is the original vesicle. In his figures (Pl. VII.) he represents only a single polar globule, but says in the text that segmentation is preceded and accompanied (!), as in most animals, by the formation of polar globules, the number, form, and volume of which are variable.

In his first studies on Anodonta, Flemming ('**74**, pp. 274–279, Taf. XVI. Figs. 10, 11, 16) traced the appearance of polar globules, but was in doubt as to whether the second of the two bodies resulted from a division of the first one, or was separately eliminated from the yolk. The globules are expelled from the yolk at the pole diametrically opposite the micropyle, and the process is introduced by the appearance of a hyaline margin which in some cases is raised to a knoblike form. This is followed by the pushing out of a rodlike projection having a conical apex.

The formation of the first polar globule is of considerable interest, inasmuch as its production is accompanied by the appearance of short pseudopoda-like projections about the apex of the conical mass. This observation stands without a parallel. The projections, although resembling pseudopodia, were never observed to execute rapid motions.

A layer of granules in the middle or under the apex of the projecting body is doubtless to be referred to the elements of the external half of the nuclear plate. (Compare *spl*, Figs. 50, 40, and 67 of Limax.) The yolk was observed to change its form periodically, during this process of elimination, from a spherical to a more flattened condition, and back again. At this time none of the yolks possessed a nucleus, but instead a "clear place" was to be seen (especially if the egg were subjected to pressure sufficient to flatten it) which was not sharply limited from the rest of the vitellus; it simply contained fewer and smaller granules than the surrounding yolk; it lay somewhat eccentric, nearer the pole where the globules were eliminated.

The polar globules persist only a short time (till the fourth segmentation); they stain more intensely than the yolk, and in this Flemming

finds reason for the belief that they take their origin from the nuclear structure, — the metamorphosed germinative vesicle and germinative dot. The second polar globule, however, cannot correspond to the whole of the clear space noticed after the formation of the first globule. In this he is unquestionably right, as the clear space, it will not now be doubted, corresponds to that portion of the first archiamphiaster which is not eliminated with the first-formed globule.

The statement by DIECK (**'74**, p. 512) that he has recognized the polar globules in the case of decapod crustaceans (Maja and Carcinus) is rendered comparatively unimportant by the inaccuracy of his ideas concerning the nature of those bodies. According to this author the elimination of polar globules — whose discovery he wrongly ascribes to Johannes Müller — is to be followed in the nemertean Cephalothrix from the first cleavage onward. They are at first large, but afterwards become smaller, in keeping with the diminution in the size of the segmentation spheres. At length they fill a great part of the space between the embryo and the chorion. This confusion of the production of polar globules with abnormal processes has already been pointed out by Bütschli. Dieck, however, observed around the germinative vesicle, and around nuclei generally, a clear zone, and during the reappearance of nuclei after each cell division he mentions that it is in this zone that the new nuclei make their appearance. The "zone" probably corresponds to an aster.

The unfortunate confusion which LANKESTER (**'74**, pp. 375, 376, Pl. XVI. Figs. 1 – 7) experienced regarding the gastrula invagination of Lymnæus was due, in part at least, to not carefully distinguishing between the polar globules and the fluid excretions which are so noticeable a feature of the segmentation stages of pulmonates.*

In a preliminary note FOL (**'74**, p. xxxiii.) makes brief mention of the

* He says: "They [Richtungsbläschen] may serve a useful purpose for the embryologist if they enable him to recognize at any subsequent period when they are present the original pole at which they made their appearance. But it must be borne in mind that such droplets of albuminous matter are occasionally extruded from eggs of the same character as those of Lymnæus at other points during later stages in the process of segmentation of the egg sphere." I believe there can be little doubt that Lankester's errors lay in considering the smooth rounded surface shown in his Fig. 4 to be the nutritive or less active pole of the egg, and in admitting the possibility of an inconstancy in the relative position of the polar globules in the case of different mollusks. Having often seen corresponding stages in Limax, I am convinced that the active pole of the egg is uppermost in his Fig. 4, as well as in Fig. 7, and that his four "large spheres" appear large only because they are very much flattened by the accumulation of fluid within. With this explanation there is no serious difficulty in understanding the process of invagination.

changes in the Pteropod egg. "At the moment of deposit one only sees in the midst of the protoplasm two molecular stars." After the escape of the polar globules, there appears a nuclear structure (which Fol still insists upon calling a germinative vesicle), which in turn soon disappears, giving place to two molecular stars. This is the beginning of the segmentation, as already described for Geryonia. "I would only add," he says, "that I have seen these stars arise in the interior of the germinative vesicle, an instant before its diappearance."

The first change in the germinative vesicle of fertilized eggs of Serpula uncinata consists, according to Schenk (**'74**^{b}, pp. 291 – 294, Figs. 3 – 8), in its becoming notched. This change is effected by a motion in the granular protoplasm of the yolk, which is directed, as larger and smaller processes, toward the centre of the vesicle. During many passive alterations of form the vesicle becomes smaller, and at length reaches the surface of the yolk. Here it is for a time distinguishable as a clear space, but finally this fades away till no recognizable trace of it is left. Meanwhile the germinative dot is eliminated, and lies between the yolk and its envelope, the latter being raised into a corresponding prominence. This eliminated dot, which is plainly stained in carmine, becomes flattened against the yolk, and finally ceases to be visible, the envelope assuming its full circular outline. The changes of the vesicle, but not the elimination of the dot, were also seen in unfecundated eggs.

Schenk adds, that one cannot be easily induced to maintain for this structure such a *rôle* as Robin ascribes to his polar globules, since in this case the fate of the germinative dot cannot be further followed, and that he has been unable to observe polar globules either in the case of Serpula or Phallusia intestinalis.

A sudden contraction of the yolk follows, and afterwards it again fills the membrane completely. The appearance of a stellate figure follows, as described at page 283.

Although principally occupied with the events which succeed maturation, Auerbach has contributed much to the understanding of this subject, for he, more than any one else, has fixed the attention of embryologists upon the nature and origin of the first cleavage nucleus, — upon the existence of two nuclear structures which, with Ed. van Beneden, I have designated "pronuclei." Auerbach (**'74**, pp. 195 *et seq.*, Figs. 1 – 7) begins the account of his studies on Ascaris and Strongylus with a stage which follows very promptly on the fecundation of the egg. By this epoch of fecundation, however, we are simply to understand a point of time at which the spermatozoa are supposed to come in contact with

the yolk, and he evidently considers the disappearance of the germinative vesicle* as a criterion of that event.

Besides the entire absence of the germinative vesicle, not the least trace of which could by any means be made visible, this stage is characterized by a temporary recession of the yolk granules from the periphery of the egg, during which the vitelline membrane is gradually formed, probably by a condensation of a superfical layer of the protoplasm. The return of the granules to the periphery is immediately followed by the contraction of the whole yolk and the contemporaneous exudation of a quantity of clear *liquor ovi.* Thus is completed the formation of the first cleavage sphere, which is throughout homogeneous.

As regards the formation of a nucleus in this first segmentation sphere, Auerbach rejected the idea that it appears either as an entirely new centrally located structure, or as a metamorphosed persisting germinative

* In the eggs of both these nematodes there is a slight deviation from the truly oval outline ; one end is slightly more obtuse than the other. The more pointed end is characterized, according to Auerbach, by several other peculiarities. It is the one which is in advance as the egg passes through the oviduct, and therefore that which is first exposed to the spermatozoa, probably also the part into which the spermatozoa penetrate. Perhaps this accounts for certain advantages which the narrow seems to possess over the more obtuse end. It is at the former that the polar globules are found ; of the two spheres which result from the first segmentation the more voluminous occupies the narrower end ; in its changes this anterior sphere slightly anticipates the hinder one, and is subject to fewer variations from the norm ; and finally, it is this portion of the egg from which the anterior part of the worm is produced.

Unfortunately, Auerbach has given no account of the place and manner in which the polar globules are eliminated from the yolk. If their constant appearance at the smaller pole of the egg could be taken as evidence that they were eliminated at that pole, — an assumption which has a certain amount of support in the less granular condition of that end of the yolk (see Fig. 27, *loc. cit.*), and in the fact that both pronuclei arise at the poles of the vitellus, — then the observation would command particular attention as showing that the almost universal relation of polar globule and first cleavage plane is not in this case maintained.

The appearance of the pronuclei at opposite poles of the egg is not easily reconciled with Auerbach's ideas, for the female pronucleus, we must now assume, makes its appearance near the point where the polar globule arises, and the male pronucleus at the large end of the egg could hardly have arisen from the influence of a spermatozoön penetrating at the smaller. In view of the fact that Bütschli (**'75**, pp. 203, 204) finds the polar globule in non-parasitic nematodes usually at the equator, though sometimes nearer the vaginal pole, and that he has observed its transportation from the place of its origin to the smaller (vaginal) pole, it is perhaps safe to infer that Ascaris and Strongylus offer no exception to the rule that the polar globule makes its appearance in the plane of the future first segmentation, in which event a sub-polar position of the globule would probably correspond to the cases of oblique segmentation so frequently observed in the nematodes.

vesicle. He traced its origin from the union of two nuclear structures, which make their appearance at the opposite poles of the egg, and, after attaining their characteristic features, migrate to its centre.

After the egg has remained some time in a homogeneous condition, these two structures simultaneously make their appearance as small clear spots close under the surface at each pole. As no difference is recognized between them, the further account is the same for both. At first irregular in shape, this spot gradually enlarges and at the same time becomes more nearly circular in outline, until, in the course of half an hour, it has attained its full size and spherical form. It is homogeneous and less refractive than the surrounding protoplasm, from which, although sharply marked off, it is not separated by a membrane. It is a cavity in the protoplasm filled with a substance probably fluid, as may be fairly inferred from the rapid motion of the nucleoli observed later in its history. After a little time there appear within this homogeneous structure from one to five nucleoli, the size of which is generally inversely proportional to their number. Just how they arise Auerbach is unable to say. At first faint, they become darker, and then larger. If numerous, they do not all appear at once, but one after the other in intervals varying from half a minute to a few minutes, and at points remote from each other.

These two thus fully formed nuclear structures now begin a slow migratory motion toward the centre of the cell, where they finally meet. Meanwhile they suffer no change of form, but the nucleoli within them often exhibit comparatively rapid changes of position. The migration is gradually accelerated. Each nucleus leaves in its " wake " an indication of the course it has pursued, in that the region traversed is less granular than neighboring portions of the protoplasm. The cause of the motion of the nucleoli the author is unable to explain.* The migration of the nuclei cannot have its cause in any power of motion inhering in the nucleus itself, nor are centres of attraction discoverable ; in fact, any explanation which presumes the protoplasm to be passive can hardly be accepted, since its passive resistance to the motion of the nucleus would cause the latter to become flattened in the direction of the motion. In short, it is the contractile power of the protoplasm which forces onward the passive nucleus, and the clear " wake " is rather the cause than the effect of this migratory operation. The activity of the protoplasm also finds expres-

* Subsequently (p. 247), he ventures the suggestion that it may be due (in case the nuclei increase in size during their migration) to fine streams of fluid (Saftströmchen) which must make their way from the protoplasm into the nuclear cavity.

sion during this period of migration in the irregular changes of form which the outline of the yolk undergoes.

Assuming that in Auerbach's observations the female pronucleus is the one making its appearance at the small end of the egg, an occasional variation from the normal method is observable; for this (female ?) pronucleus sometimes begins its migration before the male, and therefore meets it between the centre and the blunter pole of the egg. Another variation consists in the occasionally observed origin of the pronuclei at some distance from either pole, in this case, however, usually at diametrically opposite points of the surface.

The further history of the female pronucleus will be considered under the head of Fecundation.

Van Bambeke (**'76**, p. 4) has observed that in unfecundated eggs of Tinca vulgaris a spherical portion is sometimes detached during the active changes of form which the germ undergoes, and questions if these are polar globules.

The fecundated Pteropod egg, according to Fol (**'75**, p. 196), is destitute of both membrane and nucleus. It is composed of two parts, formative and nutritive, the latter being a network of protoplasm in whose meshes are found nutritive globules. At the centre of the formative part there is a star formed by granules of protoplasm arranged in straight divergent lines which extend as far as the limit of the formative part; the nutritive globules also arrange themselves in lines. After the escape of the polar globules a nucleus appears *at the centre* (au centre) of the star. The latter disappears in proportion to the increase in the size of the nucleus; and the granules and globules cease to be in line.

In his second paper on the development of Anodonta, Flemming (**'75**, pp. 109 – 118) makes some additions to his previous communications on the earliest changes of the egg. The clear spot in the yolk, although less distinct, is visible after the elimination of the polar bodies is *completed;* it therefore cannot have corresponded to the second polar globule alone. The latter, at first naked, possesses, after a variable length of time, a membrane. The appearance within it of a large, usually rough, angular, and highly refractive corpuscle, generally attached somewhere to the membrane, is probably a symptom of the death of the globules. The corpuscle and membrane stain more intensely than the contents, "so that one is reminded of a small cell with nucleus, or a nucleus with nucleolus. It would of course in the diagnosis be premature to decide by such a similarity." The polar globules are found to persist, in a shrivelled condition, much longer than was at first supposed.

As Oellacher made considerable advance on his predecessors, owing largely to the use of sections, so Flemming certainly followed rational methods in watching closely the results of staining, and by this means he came near anticipating the later discoveries of Hertwig. As the result of his own observations and those of Oellacher, Flemming insists upon a fundamental importance for the polar globules, principally on account of their constancy and their reaction with staining fluids. Of the manner in which they arise, he still leaves us in doubt. Flemming's principal objection to considering them the eliminated germinative vesicle had been the discovery, at a little later stage, when the radial figures preceding the first cleavage have already appeared (*loc. cit.*, Taf. III. Fig. 2), of a small, deeply staining body (nuclear disk of first segmentation sphere) in the middle of the yolk. Since Fol has deduced a similar nuclear remnant directly from the old nucleus of the egg (germinative vesicle), it is hard to understand, he says, how almost the whole of it (germinative vesicle) should have been eliminated. Auerbach's discoveries, however, now come to the rescue, and this "remnant of a nucleus" (Fol) may be supposed to descend from the secondary nucleus (nucleus of the first cleavage sphere), thus leaving no objection to a total elimination of the germinative vesicle. Influenced by the prevailing dogma that the formation of the polar globules is essentially a process of elimination, perhaps a *necessary* elimination, Flemming naturally raises the question if such does not precede each act of proliferation, — especially since the morphological extinction of the nucleus precedes every division of a segmentation cell, — and, in the absence of any evidence of the elimination of polar globules or their like during segmentation, answers it only by declaring that "nothing compels the assumption that every process which characterizes the *beginning* of the construction of a body must accompany each individual phase of its advance."

The preliminary account given by Bütschli (**'75**, pp. 203, 208–210) from studies on several nematodes and two pulmonate mollusks — Lymnæus and Succinea — embraces a valuable contribution toward determining the fate of the germinative vesicle and the origin of the polar globules. Soon after the eggs of non-parasitic nematodes enter the uterus, the germinative vesicle, having lost its dot, becomes less distinct, and approaches the surface of the yolk, usually at the equator, but sometimes nearer the vaginal pole. The surface of the yolk here becomes depressed to receive the clear mass of the vesicle, which lies, as it were, sunk in a pit of the granular yolk. In the case of Tylenchus, as the vesicle reaches the surface a small, round, rather dark body is pushed

out, apparently from the germinative vesicle, and it agrees very closely in appearance with the germinative dot. It is the polar globule. The vesicle appears to sink back into the yolk. In Cephalobus (Anguillula) the germinative vesicle probably spreads out its substance either *in* or *on* the clear protoplasm which at this time forms the outer layer of the yolk. Hereby, it is presumable, the nuclear substance subsequently acquires a closer relation to the spermatozoön still attached to the surface of the yolk. After the polar globules appear, they are often shoved toward the vaginal or smaller pole of the egg.

The eggs of Cucullanus are of the greatest interest. Bütschli here makes the discovery of a peculiar spindle-shaped body, which he at once homologizes with the "semen capsule" of Infusoria, — a structure arising from the so-called nucleolus. It is hard to say what structure in the fecundated egg is homologous with this infusorian nucleolus; he conjectures, however, that it is the germinative *dot*, and that consequently it is the latter which gives rise to this remarkable structure. In place of the no longer visible germinative vesicle there lies in the yolk, says Bütschli, an elongated, portly, spindle-shaped body of exceedingly interesting constitution. It is two thirds as long as the diameter of the yolk. Its middle is swollen, and its ends are drawn out into fine points. Its mass is darker than the yolk, tolerably homogeneous, often somewhat brilliant, and distinctly and finely fibrous lengthwise. Each of the fibres merges at the swollen middle part into a thick, dark, lustrous portion, which discloses a composition out of serially arranged granules. This spindle is ejected from the yolk, on the surface of which it is seen to lie. But what becomes of it he is unable to say. From a comparison with the polar globules of the snail's eggs,* Bütschli conjectures that the two structures (spindle and polar globules) are identical, and that the dark granules and fibres in both cases correspond, although no intermediate stage between the two was discovered.

Concerning the origin of the new nuclei of the first segmentation sphere, Bütschli is able to confirm in part Auerbach's observations, but also to add observations not directly reconcilable with his theories. The nuclei arise in the clear protoplasm of the periphery of the egg, which is accumulated at certain points. It is not to be concluded that the nucleus is the result of the metamorphosis of the protoplasm itself, it is formed rather from the material of the germinative vesicle. In Cephalobus rigi-

* These appear in the form of two more or less spherical bodies, connected by a stalk, and containing each a disk of granules, the individual grains of each disk being joined to the correspondingly situated grains of the other by pale delicate filaments.

dus, Rhabditis dolichura, Diplogaster, and Succinea, there appear only two such nuclear structures; in Cephalobus, at the poles, but not always at the same time; in Rhabditis, one at the vaginal pole, the other at the point of the surface where the vesicle disappeared, whether the equator or nearer the vaginal pole. The migration and union of these two nuclei, except in Cephalobus, is less regular than Auerbach represents it to be. The amœboid motion of the yolk at this time is sufficient to explain the migration, but not the coalescence, of the nuclei. In the cases of Rhabditis, Cucullanus, and Lymnæus there are, however, *more than two* nuclear structures, — from three to eight, or even more, according to the animal. In Cucullanus these arise close under the surface at points remote from each other; in all cases they are at first quite small, and successively unite till a single nucleus results. In the author's opinion the formation of the nucleus of the first segmentation sphere by the union of *several* nuclear structures, since it is of wide-spread or general occurrence, refutes Auerbach's idea that the whole process results from fecundation taking place at a definite pole of the egg, and the same is shown even more conclusively by the fact that the nuclei of later generations also arise from the union of several nuclear structures.

Goette (**'75**, pp. 20 – 22) has described the regressive metamorphosis of the germinative vesicle in the case of Bombinator igneus. After approaching the upper (dark) pole of the yolk, it is found to have suffered diminution of volume, so that it no longer fills completely the cavity in the yolk which it once occupied. The remaining space of this cavity is filled with a clear fluid. At a later stage there appears at the centre of the dark pole, therefore directly over the vesicle, an irregular yellow spot, which is found, on making sections, to be due to an interruption or obliteration of the pigment layer, which thus allows the uncolored yolk to come to the surface. It is found at the same time that the cavity about the shrunken vesicle has disappeared. Goette concludes that the former appearance is caused by the escape of the clear fluid of this cavity, whereby the integrity of the pigment layer is interrupted. There are still some traces of the germinative dots and of the membrane of the vesicle. A little later there is to be found in place of the vesicle only an exceedingly fine-granular substance without definite limits. All this takes place before the eggs are laid, therefore independently of fecundation.

In freshly laid eggs certain changes in the region of the yellow spot are to be observed. But only a few of the fresh eggs exhibit all the phenomena. The middle of the spot is often depressed, and sometimes

presents the same appearance as the button used to upholster a cushion. After a short time the button disappears, and in its place there remains a hole such as arises in making a thrust into a doughy mass. This hole — there may be several of them — may remain till segmentation ensues, or it may disappear earlier. The yellow spot ultimately vanishes.

Is not this "button" identical with the spheroidal structures discovered in fishes by Oellacher, and considered by him as the representatives of the polar globules?

Of the results attained by BÜTSCHLI (**'75**[a], p. 430) in a second preliminary paper, I will here call attention only to the modification of his views concerning the source of the spindle-shaped body. He now believes it must be considered the metamorphosed *nucleus* rather than nucleolus, and that in the light of this his previous conclusions are to be correspondingly modified. Further details of his paper are given at page 289.

SELENKA (**'75**, p. 444) compresses into few words his observations on Phascolosoma elongatum. Some time after fecundation the germinative vesicle disappears, the yolk contracts, and there is pressed out a drop of protoplasm, which he thinks may be the remnant of the "cell nucleus," — perhaps excrement of the egg. I think it is without doubt a polar globule.

FOL'S (**'75**[a], pp. 104 – 108, 198, Pl. I. Figs. 3, 4, Pl. VII. Fig. 2, and Pl. VIII. Figs. 1 – 3) illustrated paper on the development of Pteropoda, beside giving a summation of results (p. 198) in the words of his preliminary paper, furnishes additional facts of interest, and affords by the figures a better means of judging accurately the nature of his observations.

He says the centre of the delicate star which occupies the middle of the formative part of the egg at the time the latter is laid, is not occupied, as one might expect, by a corpuscle differing from the surrounding stroma; the granules composing the star also occupy its centre. No activity is to be attributed to the granules themselves; they are only the landmarks, as it were, of the intimate molecular movements of the protoplasm which one is unable to observe directly. Some minutes after the egg is laid this *star begins to elongate* in the direction of the long axis of the egg. It soon *divides* into two stars, of which one continues to occupy the centre of the protoplasm, while the other reaches the surface in the middle of the protoplasmic area. This point then becomes elevated as a small nipple, and separates itself from the yolk as a spherical globule, for which Fol adopts the term *corpuscle excrété*, or *corpuscle de rebut*, as better reflecting than does the term "Richtungsbläschen" its entire want of significance in the subsequent development. This polar globule

divides into two, usually unequal portions. The escape of two globules, one after the other, was never observed.

I think these statements about the division of the polar globules would bear the confirmation of renewed observations.

It is the central part of the star which escapes as a polar globule, and the interior of the globules becomes differentiated into protoplasm and nucleus.

This last statement, like Flemming's, appears almost to forestall the work of O. Hertwig, but it will be observed, after all, that there is a wide difference between the differentiation of an excreted corpuscle into nucleus and protoplasm *after* it is expelled from an egg, and the process of division by which the polar globule is really formed.

That which remains of the *peripheral* star, continues Fol, is little by little mingled with the protoplasm; but the *other* star, which is more extensive, always occupies the centre of the protoplasm; *its* centre becomes homogeneous, and the star gradually disappears. There arises at the centre of this star a homogeneous corpuscle of slightly less refractive power than its vicinage, whether a vesicle or a more or less solid body is hard to say. Soon two or three similar structures make their appearance by the side of the first, and from the fusion of all results the "germinative vesicle" or nucleus of the fecundated egg.

Fol figures only one case (Cleodora, Pl. VII. Fig. 2) in which there is more than a single homogeneous corpuscle of this nature, and only *two* are indicated there, one of which we may safely assume is the male pronucleus. From a comparison with his other figures of early stages of Cymbulia, I am almost certain that the nuclear structure (v) represented as occupying the centre of the deeper star in Fig. 2 of his eighth plate is not the female, but the *male* pronucleus, — in other words, that the aster of which it is the centre has no such genetic connection, as Fol assumes, with the remnant of an aster (a) lying under the polar globule. There are several reasons why it is more consistent to assume that Fol has confounded these two structures, than to grant that the deep star of the figure referred to is one which took its origin from a division of the first aster. Special attention had not been called to the different origin of these nuclear structures, even by those who had observed them; again, I know of no parallel case where the *female* pronucleus lies so much nearer the vegetative than the animal pole of the egg; and, finally, I believe this assumption explains why Fol allows this nuclear structure to occupy the *centre* of the stellate figure, a thing which can hardly be predicated of the female pronucleus and its aster before the disappearance of its rays.

The main objection to this view is the fact that no other complete aster, and no indication of another nuclear structure which might be the *female* pronucleus, is made to appear in this figure. I can only assume that the latter was overlooked.

As the ovarian egg of Toxopneustes lividus approaches maturity it contains, according to O. Hertwig (**'75**, pp. 349 – 358), a large spherical germinative vesicle with nuclear membrane derived from the protoplasm, clear contents, and a spherical germinative dot of constant size (13 μ) and homogeneous structure. The dot is deeply stained in carmine, the clear contents only feebly. The two are designated as nuclear substance and nuclear juice respectively. Besides these, a network of fine pale fibres stretches through the vesicle, for whose membrane it forms a lining, and is especially concentrated about the dot. In the mature egg found in the oviduct, on the other hand, the germinative vesicle has disappeared without leaving a trace, but there exists a small clear spot which before was not present. The latter is spherical, and 13 μ in diameter. Intermediate stages lead Hertwig to the conclusion that the vesicle is expelled from the egg, that it at first lies in a lenticular depression of the yolk, but afterwards becomes flattened, that its membrane is dissolved and its contents become disintegrated, and probably that it is subsequently absorbed by the yolk. He believes, however, that the germinative dot persists without change, and either actively or passively comes to occupy a position in the yolk, as the clear spot already alluded to. The assumption of the identity of the germinative dot with the clear spot (Eikern) is supported in the author's opinion by equality of size; and by the facts, that both consist of a tolerably firm homogeneous substance without enveloping membrane; that both are stained intensely in carmine and blackened in osmic acid; that the disappearance of, or any change in the dot, could not be observed, nor any steps in the formation of the Eikern; that both structures are never met in the same egg, and never are both absent; and, finally, that the Eikern first appears near the metamorphosed germinative vesicle, while the dot is last to be seen in immediate contact with the surface of the yolk. The necessity of a migration from the vesicle into the yolk cannot be an objection to the identity, since nucleoli have often been observed in amœboid motion.

In a general discussion of the topic, Hertwig attempts to harmonize conflicting views, or at least to explain the reasons of such differences. The testimony of those who describe a regressive metamorphosis and disappearance of the germinative vesicle must be accepted as valid for the cases described; the positive assertions of those who claim that the

vesicle divides and gives rise directly to the nuclei of the segmentation spheres are, however, capable of another explanation, and such cases are therefore not to be considered as exceptions to the ordinary course of events. The trouble lies in the confounding of two entirely different morphological structures, — the germinative vesicle and the egg nucleus (Eikern). The former possesses a firm membrane, fluid contents, and a compact germinative dot; the latter is without membrane, homogeneous, and without a nucleolus. As it is the latter which has been observed to divide, Hertwig concludes that one may already affirm with certainty that *at the maturity of the egg the germinative vesicle as a morphological structure perishes.* To say how the "Eikern" originates is more difficult.

A cardinal point is touched by the question, Does the egg exist at a definite stage of its development as an enuclear yolk mass, — a cytode? The evidence furnished by those who maintain that such is the case, Hertwig endeavors to weaken, by showing that, although a germinative vesicle could hardly be overlooked, it would be quite easy to pass unnoticed so small and little differentiated a structure as the germinative dot, — the more, since anything like a satisfactory conclusion can only be reached by having recourse to various reagents, and especially to methods of staining. His own observations have shown conclusively that in some apparently very carefully studied cases a nuclear structure was really present, and had been overlooked by his predecessors. Moreover, in most cases the *possibility* of the persistence of the germinative dot had not been sufficiently impressed upon the observer to make the observations certain on this point. Even in those cases (Auerbach and Strasburger) where a direct observation of the origin of the new nucleus is claimed, it is not impossible that a very small germinative dot may have remained unobserved and been in reality the initial stage of the supposed new structure. The evidence, then, in favor of a new origin for the "Eikern" is insufficient; for, on the one hand, it is not established that the egg passes through the cytode condition, and, on the other, the positive statements that the nucleus is a new creation are capable of another explanation. Another error is coupled with this; namely, that fecundation is the cause of the disappearance of the germinative vesicle and of the formation of a new nucleus. "Der Schwund des Keimbläschens und die Entstehung des Eikerns sind vielmehr Vorgänge, die einzig und allein mit der Reife der Eier zusammenhängen und die Befruchtungsfähigkeit derselben herbeiführen."

Finding no entirely insurmountable obstacle in the literature, and supported by his own observations, he draws the general conclusion that "in

the whole animal kingdom the 'egg nucleus' of the mature egg capable of being fecundated arises from the dot of the germinative vesicle which [latter] is dissolved."

In a paper on the development of fresh-water pulmonates, Rabl ('75, pp. 197, 198, 223) adopts Haeckel's view of the phylogenetic significance of the disappearance of the germinative vesicle; namely, that it is evidence that the earliest ancestors of the Gasteropoda, as of all other living organisms, were of the simplest possible structure. The polar globules emerge from the yolk on account of its contractions during the first segmentation, and are usually two in number, the first one being the larger. Rabl entertains peculiar ideas concerning their physiological signification. Since, after a period of quiet, they are uppermost, he concludes that the pole at which they appear is the specifically lightest part of the egg, and that it is safe to assume, inasmuch as they are thus interposed between the egg and the envelope of the albumen, that their function is to *protect the egg from pressure.* For this reason one must consider these structures protective organs of the embryo acquired through adaptation to the method of unequal segmentation.

In Helix at the time of the disappearance of the germinative vesicle, or soon after, there emerge from the yolk, according to Von Jhering ('75, pp. 303, 304), from one to three polar globules. Whether the vesicle simply perishes, or is ejected, whether or no there is a connection between it and the polar globules, cannot be determined on eggs so unfavorable for study. The formation of the globules is proof for the author of the existence of a vitelline membrane (Taf. XVII. Fig. 2. *d*).

Without contributing any personal observations which bear immediately on the early stages of the egg, Haeckel ('75, pp. 421, 426, 434, 435, 446, 480–483) utilizes the preponderating evidence in favor of the disappearance of the germinative vesicle in support of his theory of the palingenetic significance of the cytode stage of the egg, as a "Rückschlag der einzelligen Urform in die primordiale Stammform des Moneres." If this atavistic return to the cytode condition should be established for only a part of the animals, but fail for the remainder, then the development in the latter cases would have to be considered as a *cœnogenetic* process.

Ludwig ('75, p. 210) was unable to detect any such details in the early stages of the egg of Chætonotus as have been described by Auerbach and others, and therefore was only able to say that the germinative vesicle disappears entirely while the yolk undergoes contractile changes.

I should not have occasion to call attention here to a paper by SEMPER (**'75**, pp. 4 – 13), in which he discusses the origin and nature of the so-called Testazellen of ascidians, were it not for his attempt to identify these structures with the polar globules of other animals, — the less occasion, as his observations relate principally to artificial productions which do not contain nuclei (although the same is true in his opinion for the normally produced "Testazellen") and are only entitled to the name "Testa*tropfen*." The reasons given to establish the identity alluded to have proved to be unfortunate, as Whitman (**'78**[a]) has already pointed out. Nor can Semper's claim be maintained, that the polar globules "first make their appearance at the moment of segmentation," * or that they "are enuclear"; nor is there any evidence to prove that they are capable of moving themselves in an amœboid fashion around the embryo. Even though a change in the contour of these globules has often been observed, and the existence of *rigid* processes which resemble pseudopodia has been demonstrated by Flemming, it is far from proving the polar globules endowed with the power of making excursions on their own account about the embryo. As Kupffer (**'70**, pp. 123, 124, and **'72**, p. 366) has shown, the "Testazellen" appear while the germinative vesicle is still intact, and this is not objected to by Semper, who admits that they arise, not from the nucleus, but from the yolk. The genetic connection of the polar globules with the germinative vesicle therefore forbids the comparison which he has instituted. But when Semper, partially recognizing the possibilities of such a genetic relation, in a foot-note substitutes for an "Uebereinstimmung in fast jeder Beziehung" a *physiological* comparison between these two sorts of bodies, he is no longer defending the view already promulgated, — a view, it is to be observed, which he endeavored to establish with *morphological* arguments, — but is really supporting new ideas. The physiological *rôle* which Semper discovers in these bodies is "the detachment of a hitherto integral component of the egg-cell, in some manner a defecation of the same, an elimination of substance apparently useless for the approaching processes."

In a preliminary paper on early stages in the development of the rabbit, ED. VAN BENEDEN (**'75**, pp. 690 – 693, 695 – 700) signalizes the existence of two or three small round bodies (*pseudo-nucléoles*) and a

* P. S. — The recent statement by Brooks (**'80**, p. 79, Pl. I. Figs. 3, 4) that the polar globules in Physa *arise during segmentation* will also probably be found to be inaccurate, for it is not consistent with what is known of the nature of these globules in all other investigated animals.

granular substance (*nucleoplasma*) in the germinative vesicle, in addition to the nucleolus and a clear liquid. The granular substance often assumes the form of a network in the growing egg. At maturity the vesicle, instead of being central, becomes superficial, takes an ellipsoidal form, and then becomes more and more flattened against the zona pellucida. The vitellus is now composed of a medullary mass, and a cortical layer which becomes clear at the contact of the vesicle. Clear protoplasm is accumulated around the vesicle in the form of a biconvex lens, — *la lentille cicatriculaire,* — which depresses the medulla. As soon as the germinative vesicle comes in contact with the zona, the nucleolus joins the membrane of the vesicle, against which it is flattened and with which it unites ; its plastic substance spreads out into a plate with, at first, a median thickening, — *plaque nucléolaire.*

At the same time the membrane of the vesicle thins out, especially where it is in contact with the cicatricular protoplasm. It is probable that the substance of the membrane is attracted toward, and unites with, the nucleolar plate. The nucleoplasm and pseudo-nucleoli give rise to a mass of granular substance, — *corps nucléoplasmique.* The liquid and limpid contents of the vesicle mixes with the cicatricular protoplasm upon the rupture of the membrane of the germinative vesicle. At the same time the nucleolar plate, by virtue of its inherent contractility, is amassed into a body having sometimes the form of an ellipsoid, often that of a lens, or of a calotte, — *corps nucléolaire.* At the moment the germinative vesicle disappears, the directive bodies are eliminated. There are two of these, but they are unlike both in composition and signification ; one is the *nucleolar* body, the other the *nucleoplasmic* body. The former is stained in picrocarminate of ammonia, the latter is not. The cicatricular lens becomes granular, and thus indistinguishable from the cortical layer of the yolk. With the disappearance of the germinative vesicle begins the retraction of the vitellus, which consists in the expulsion of a transparent *liquide perivitellin,* and is accompanied by amœboid movements. Subsequently the vitellus resumes its spherical form, and no division into cortex and medulla is visible. In this cytode state the egg is entitled to Haeckel's designation, "monerula." All the preceding changes are independent of fecundation, and are connected with the maturation of the ovum. In the case of the rabbit they are accomplished within the ovary.

Although a portion of what is said in his chapter on the "Formation of the first Embryonic Nucleus" pertains to another part of the present review, I shall give it in this connection. Shortly after fecundation

the substance of the vitellus consists of three layers, — superficial, intermediary, and central. The second is coarsely and irregularly granular, and more opaque than the other two; the central is clearer, but uniformly granular; the superficial is almost homogeneous, very refringent, and contains only punctiform granulations. At a point of this outer layer a thickening occurs, and in this point appears a small, round body, which is destitute of granulations and resembles a vacuole; but in osmic acid this so-called vacuole darkens and assumes a gray tinge, while the vitellus is colored brown. This is the *pronucleus périphérique*. This sinks deeper into the yolk, at the same time becoming larger, and there appear within it numerous very refringent corpuscles of variable size which resemble nucleoli. In the "central mass" of the egg there appear simultaneously two or three small clear irregular masses, which directly unite into a body with bunched (bosselé) surface. This occupies from the first the centre of the egg. It is called by Van Beneden *pronucleus central*. It differs from the peripheral pronucleus in being considerably larger, and in having a less distinct contour. The two approach till they touch each other at the middle of the yolk. The peripheral pronucleus is *spherical*, and its contour is regular. The central has the form of a calotte or of *a flattened crescent with blunt horns*,* its concavity being moulded upon the peripheral pronucleus, from which it is at first separated by central protoplasm sometimes containing several voluminous and refringent granules. In most of the eggs, however, the pronuclei touch or are separated by only an imperceptible layer of vitelline protoplasm. The convex face of the central pronucleus is sometimes regular, sometimes lobed, and occasionally divided into two parts in such a manner that there are three conjoined clear bodies. The substances of central and peripheral pronucleus are optically alike, and both exhibit rounded refringent corpuscles of variable size, — the nucleoli.

The peripheral pronucleus increases rapidly in size, but preserves its spherical form. The central diminishes in volume. They become much less apparent, and at length there exists only one nucleus formed at the expense of the two. Whether they fuse, or one is developed at the expense of the substance of the other, the author is unable to say. This nucleus has an irregular form, indistinct contours, and is composed of a homogeneous substance in which nucleoli are not distinguishable. From the time the pronuclei approach each other in the centre, the vitellus

* These eggs were treated with 1% osmic acid, put two or three days in Müller's fluid, and then mounted in glycerine.

presents a radiated appearance, which the author does not, however, further describe. These latter stages are exhibited by unsegmented eggs found in company with eggs already divided into two segments, taken from the middle or from the lower half of the oviduct.

From all this it is concluded that the first embryonic nucleus is developed at the expense of two pronuclei, one peripheral, the other central. As the spermatozoa have already been shown to become mingled with the superficial layer of the yolk, it is probable that the peripheral nucleus is formed, at least partially, at the expense of the spermatic substance. "If, as I think, the central pronucleus is formed exclusively from elements furnished by the egg, the first nucleus of the embryo will be the result of the union of male and female elements." This latter, however, he expressly states, is only an hypothesis.

In the description of the polar globules there is a notable deficiency. Although a fundamental difference is maintained for the two globules, we are not informed of the order in which they make their appearance. In the present state of our knowledge it can hardly be granted that there is any such fundamental distinction between the two as Van Beneden maintains; it would nevertheless be interesting, and possibly not without important significance, to know if in any case there is a noticeable difference in the intensity with which these globules respond to the influence of reagents, especially of staining fluids. For Limax I can only report, without having directed particular attention to the point during my observations, that I have not noticed any constant difference, though I should not wish to assert positively that a more careful study, limited to this single point, would not teach otherwise.*

In another direction the studies of Van Beneden are of especial interest. I refer to the condition of the two pronuclei, which he has described as a moulding of the central (female) upon the peripheral pronucleus. The possibility of this condition having been produced by the influence of the hardening reagent (osmic acid), does not seem to have occurred to the author. The more I reflect upon it, the more it seems to me this condition may be attributable to the same cause as that which produces similar conditions already described for eggs of Limax treated with the

* There are, however, some reasons why it would be difficult to reach entirely convincing evidence on this point. A comparison between the first globule of one egg and the second of *another* would have to deal with unknown individual differences in the eggs, and other possible differences of conditions; while in a comparison between the two globules of the same egg one could not ignore the possibility of changes (of degeneration) in the older of the two globules which would seriously diminish the value of such comparisons.

same reagent. I will therefore state somewhat more explicitly my conception of how this condition may have been brought about. The *sudden* loss of a quantity of fluid would not be covered by a gradual and uniform shrinkage of the whole nucleus, but would be followed by a giving way of the wall at its weakest point. There is certainly considerable evidence tending to show that *that* portion of either pronucleus which is directed toward its mate is the one which first shows signs of losing its integrity (compare Limax, Fig. 70), — is therefore, we may assume, least capable of withstanding external pressure. It would not be surprising, then, to find either of the nuclei yielding first at this point. There are manifest reasons (their close approximation) why the apposed faces would not yield by a movement in opposite directions; the one which, from any cause, exhibited the earlier or stronger tendency to such a change, would entail in its action the corresponding face of its mate. The latter would thus fill the depression caused in the surface of the former. *Where* the depression in the latter nucleus to balance this out-pushing should occur, would depend, aside from the point of least resistance, upon the *direction* already given to its substance by the process just described. Thus the pole opposite the eminence already formed would be the point to yield. Although described as successive, these events may nevertheless be conceived as simultaneous in their occurrence. Such a conception would, it seems to me, be quite feasible in explaining the shapes presented by the pronuclei in the case of Limax, and at the same time offer a possible explanation of the apparent absence of nucleoli. In the case of the rabbit, as described above, however, it is only the central pronucleus which thus suffers an involution. This appears at first to offer an objection to the above explanation, but when one reflects that the central pronucleus is described as being much larger and less conspicuous than its mate, it is possible to believe that this alone is enough to indicate that the central pronucleus may lose much more fluid than does the peripheral. A more serious obstacle appears to lie in the fact that here the nucleoli probably remain visible notwithstanding this condensation. Moreover, these are not occasional but constant conditions in the approximated pronuclei of rabbits' eggs, so far at least as can be inferred from the description. If I had been able to reproduce these conditions, even with other reagents than osmic acid, I should be less confident that they represented relations not normal for living pronuclei.

SCHULZE'S (**'75**[c], p. 267) excellent paper on the development of Sycandra, unfortunately, does not afford much insight into the changes which overtake the germinative vesicle. He believes it disappears, but has not seen any polar globules.

Robin ('**62**, '**62**$^{b-f}$) deserves great credit for having a long time ago called especial attention to the changes which the egg undergoes previous to cleavage. The changes within the cell were, however, incompletely observed, and, though still (1875) maintained, in many particulars fundamentally wrong. In his more recent memoir on the development of the Hirudinea, Robin ('**75**, pp. 26 – 79) has reproduced with slight additions these earlier papers. The description in the one on the formation of the polar globules is of particular interest, as it contains an allusion to a phenomenon occasionally seen in Limax, but not hitherto noticed in other animals. Robin says ('**62**^{d}, p. 156, Fig. 8, and '**75**, p. 35, Fig. 10), in describing the formation of the first polar globule in Nephelis : "At the same time (i. e. during the constriction which rounds off the polar globule) the clear space of the vitelline mass diminishes more and more, until the separation is complete, *or a plane of division* is produced at the junction of the vitellus and the part which is narrowed into the form of a pedicel. This plane of division presents the aspect of a slender grayish or blackish transverse line, and establishes a complete separation between the vitellus and its prolongation, which then constitutes a distinct polar globule." Although this plane does not (in his figures) quite correspond in position to that which in Limax I have ventured to call the cell plate, I have little doubt that it is really the same thing. It seems also in Nephelis to be only an *occasional* method of finally terminating the direct connection of yolk and polar globule.

Robin has in his recent work ('**75**, pp. 97 – 105) given a detailed account of the changes which accompany the formation of "polar rings" in Clepsine, or of such as can be seen on living eggs. As this does not very essentially differ from the account given by Whitman ('**78**^{a}), I omit a review till it can be given in connection with the observations made by Whitman on the accompanying internal changes.

The first chapter of Balfour's ('**76**, pp. 378 – 387, Pl. XV. Fig. 1, and '**78**, pp. 1 – 9, Pl. I. Fig. 1) Development of Elasmobranch Fishes is devoted to the ripe ovarian ovum. Here he concludes that observations in the case of Raja batis, as far as they go, tend to show that the thick membrane of the germinative vesicle is expelled, but that the contents of the vesicle become mingled with the surrounding yolk. He explains (p. 8) how, under certain assumptions, a "consistent account of the behavior of the germinative vesicle throughout the animal kingdom" may be framed. "The germinative vesicle, usually before, but sometimes immediately after impregnation, undergoes atrophy, and its *contents* become indistinguishable from the remainder of the egg. In those cases in

which its membrane is very thick and resistant, — e. g. Osseous and Elasmobranch Fishes, Birds, etc., — this may be incapable of complete resorption, and be extruded bodily from the egg. In the case of most ova it is completely absorbed, though at a subsequent period it may be extruded from the egg as the Richtungskörper. In all cases the contents of the germinal vesicle remain in the ovum."

In a paper on the germinative vesicle and first embryonic nucleus Ed. van Beneden (**'76**[a], pp. 38–76, and **'76**[b], pp. 153–178) gives a very minute account of the disappearance of the vesicle in Asteracanthion rubens. Much of this paper is taken up with a comparison of his results in this case and in that of the rabbit, but more especially with a comparison of his own results and those reached by Hertwig in the study of Toxopneustes. We learn here (**'76**[a], p. 40) for the first time definitely, that in the rabbit the nucleoplasm with the pseudo-nucleoli forms the *second* of the two polar globules. He expresses here more positively his conviction that the substance of the central pro-nucleus is absorbed in an endosmotic way by the peripheral pronucleus.

The vitellus of the Asteracanthion egg is composed of a clearer, less granular cortical layer with radiated striations, and a central mass which occupies two thirds the diameter of the egg. In the germinal vesicle are to be distinguished the parts already described in the case of mammals: a nuclear membrane enclosing a transparent and perfectly homogeneous liquid; a germinative spot formed of a very refringent and brilliant substance enclosing a variable number of clear vacuoles; a reticulum of a finely granular substance (*nucleoplasma*) starting out from the germinative spot as a centre and embracing in its substance the pseudo-nucleoli. The latter vary in size and in number (from 8 to 15), and may be spread through the whole vesicle, but usually are situated in the vicinity of the true nucleolus, from which they differ in being much less refractive.

Of nuclei in general Van Beneden holds, that the young nucleus is formed of homogeneous matter, *essence nucléaire*. When it enlarges, the nuclear essence becomes united with a substance (*suc nucléaire*) taken from the protoplasm of the young cell. The *substance nucléaire* which results from this union constitutes the body of the nucleus. The membrane of the definite nucleus and the nucleoli are unmodified remnants of the primitive young nucleus; they are formed exclusively of nuclear *essence*. When a nucleus is about to divide, the nucleoli and the nuclear membrane dissolve in the nuclear *substance;* for this reason the contour of the nucleus becomes very indistinct and the nucleoli disappear. After

this solution a complete separation ensues between the nuclear *essence*, which goes to form the equatorial zone, and the nuclear fluid (*suc*), which is repelled to the poles of the nucleus. After the division of the zone into two nuclear disks which are to become the new nuclei, this nuclear fluid loses itself in the body of the cell. The vacuoles of the nucleoli are only the result of the momentary union of certain parts of the nuclear *substance* with the nuclear fluid.

In the case of the nucleus of the central cell of Dicyema, the use of osmic acid followed by picrocarminate results in giving the nuclear *substance* a rose color, the nucleolus and membrane a bright red, and in leaving the reticular substance unstained.

In the disappearance of the germinative vesicle of Asteracanthion, which takes place in exactly the same manner whether the eggs are fertilized or not, the nucleoplasm and pseudo-nucleoli first disappear, then the dot and the contour of the vesicle become paler, the vacuoles of the dot become confluent, and the surface of the dot gradually becomes lobed and finally breaks up into a large number of fragments which separate and spread through the whole vesicle. These fragments increase a little in volume, become less refractive, and finally cease to be visible. Some seconds after this the membrane of the vesicle becomes ruptured on the side toward the centre of the egg and parts of its contents escape; the membrane finally dissolves away, and there remains only a clear spot, with ill-defined and increasingly irregular contour. The spot diminishes in size till it vanishes. Van Beneden saw the polar globules * "formed under his own eyes," but is unable to give any account of their real origin. All his observations appear to have been made on living eggs, which accounts for his having overlooked many facts.

The principal conclusions of VAN BAMBEKE (**'76**, pp. 99–117), reached by the study of Pelobates, Triton, and Axolotl, have already (p. 389) been stated. It only remains to add that he never observed the formation of polar globules, but in eggs of the Axolotl, immersed in alcohol immediately after fecundation, he discovered the existence of a whitish spot at the niveau of, and all around, the *fovea germinativa*, caused by a superficial layer of probably coagulated albuminoid matter, which gradually thinned out toward its periphery. This layer presents in section (Pl. II. Figs. 5 and 6) a striation perpendicular to its sur-

* By a double error of translation "corps directeurs (globules polaires)" appears in the English translation in the absurd form of "*distinctive* bodies *and* polar globules."

face, and is strikingly similar to the veil-like layer seen by Oellacher in the unimpregnated egg of the trout. Van Bambeke, however, objects to Oellacher's interpretation of this layer, as far at least as regards batrachians, since in the eggs of the latter the envelope of the germinative vesicle never presents the characters pointed out by Oellacher for the trout's egg.

Greeff (**'76**, pp. 34, 35) gives a short preliminary notice of early stages of Asteracanthion rubens. The mature egg has two envelopes: "Gallertzone" and "Eihaut." The yolk is composed of a homogeneous clear "Grundsubstanz" and two kinds of granules. The germinative vesicle is clear, and has a distinct membrane; the germinative dot is more compact, and embraces small round vesicles variable in number and size. Delicate filaments, stretched through the space of the vesicle, are beset with pearl-like nodules, and exhibit spontaneous motion and branching. After fructification — or without it, if the egg remains a time in pure sea-water — the vesicle disappears, but the *germinative dot persists.* This, in the fecundated egg, *migrates like an amœba through the yolk.* The latter assumes a radial appearance about the now centrally located germinative dot. The polar globule appears with the first-segmentation constriction, or even later. (!) Nothing of a spermatic nucleus was seen, nor are the polar globules to be connected with spermatozoa.

Giard (**'76**[a], pp. 233, 234) traces early changes in the egg of one of the sedentary annelids (Salmacina Dysteri) as follows. After fecundation the germinative vesicle ceases to be visible, and one observes the appearance of a circular, finely granular spot at the surface of the egg, over against which there are two polar globules. The spot in turn disappears and the egg suffers a constriction *less* (?) pronounced on the side where the spot was situated.

Besides the entire absence of the germinative vesicle in the excluded eggs of the spider (Philodromus), Ludwig (**'76**, pp. 473, 479) contributes nothing which concerns us in this connection.

Stecker (**'76**[b], p. 125) also reports for the eggs of Chthonius that the germinative vesicle — after becoming more elongated, as seen in his figures — entirely disappears, and near the place where it perishes a brown round spot, composed of the "coarser granules" of the protoplasm, makes its appearance. This undergoes division with the subsequent total segmentation of the egg.

In a chapter first introduced into the second edition of his book on "Zellbildung," etc., Strasburger (**'76**, pp. 297 – 305, Taf. VII., VIII.) discusses at some length the question of the fate of the germinative

vesicle. Led by Hertwig's studies on Toxopneustes to a re-examination of this topic, he finds the opportunity, with improved methods of treating the eggs of Phallusia, to correct the statement in the first edition to the effect that the mature eggs are altogether destitute of a nucleus. By employing osmic acid on alcoholic preparations, he is able to demonstrate the existence of a structure (Taf. VIII. Figs. 2, 3) which he designates with Hertwig as Eikern. "Dieser Eikern," says Strasburger, "liegt hier der Hautschicht nicht * an, ist derselben oft sogar angedrückt, ausser dem aber von einer helleren Zone umgeben, die aber nicht scharf gegen das angrenzende Protoplasma abgeschieden ist." I question the accuracy of the conclusion to which Strasburger here arrives. No one has hitherto called attention, I believe, to the possibility of any other interpretation for these figures than that which Strasburger himself gives. Nothing can be further from my purpose than to cast doubt on the persistence of nuclear substance in the egg. It is quite another question if the flattened lens-shaped body represented in Strasburger's figures 2 and 3 (Taf. VIII.) is really this remaining nuclear substance. The interpretation which I am inclined to give these figures is quite different, and of some importance as bearing on *the existence of polar globules in the Tunicata.* If, as I have no reason to doubt, Bütschli was right in saying ('**76**, p. 384) that hitherto nothing had been observed concerning polar globules among Tunicata, this will, in my opinion, be the first evidence tending to show that such bodies are really produced in that group of animals; for I suspect that these figures represent a stage just prior to the formation of a polar globule. This explanation occurred to me when comparing Fig. 50 (Limax) with Strasburger's figures. Much the most conspicuous part of Fig. 50 is the areal corpuscle (aa') of the peripheral aster. Were the egg for any reason to become less transparent, it is readily conceivable that all the other parts might become indistinguishable and still leave this flattened oval structure visible, and the surrounding radial system would then appear simply as a less granular or clear zone, a condition of affairs, in other words, which is completely realized in Strasburger's figures. The features which make it possible for me to maintain the identity of these two structures may be stated as follows: —

(1.) The *shape* of Strasburger's "Eikern." — I know of no case in which the egg nucleus (female pronucleus) exhibits such a remarkable form, — apparently that of a very much flattened biconvex lens of which the thickness is (in Fig. 3) not over one fourth its diameter.

* This is evidently a typographical error for "dicht."

When any considerable deviation from the spherical form of the female pronucleus is noticeable, the latter will, I think, be found to be lengthened rather than shortened along the polar axis of the egg. On the other hand, this flattened condition is quite constant for the corpuscle occupying the centre of the peripheral star of an archiamphiaster.*

(2.) The *position* of the "Eikern" is such as to make quite improbable the interpretation given by Strasburger. It is true that, if a polar globule is not formed here as in the majority of animals (viz. by the division of a spindle body), then we have no right to assume the fulfilment of all the conditions which obtain in such a process; but granting for the moment that here as elsewhere a polar globule is thus formed, then the female pronucleus could hardly have the position close to the surface, as given in the figure, much less the position indicated by the words "pressed against the Hautschicht." The constriction which separates the two unequal cells — polar globule and yolk sphere — divides the spindle figure approximately in the middle. The interzonal filaments must be reduced to zero in order to allow the lateral zone of thickenings to form a new nucleus close to or in contact with the thin cortical layer of the yolk. There is abundant evidence that the zone of thickenings which pertains to the polar globule forms a nucleus in, or *beyond*, the centre of the polar globule, therefore at some distance from its last formed surface;† and I recall numerous illustrations which place the corresponding parts of the half-spindle remaining in the yolk at an equal or greater distance from its surface,‡ — none which place the female pronucleus so close to the surface as in the case of Phallusia, if, perhaps, I make an exception of the case of Hippopodius figured by Hertwig.§

If, on the other hand, one takes into account the migration of the spindle as described for Limax, the fact that the corpuscle, *a a'*, occupies a position close to the surface in the stage represented by Fig. 50, and that it ultimately comes to be fused to the apex of the polar globule (Fig. 63), then the interpretation I have given to Strasburger's observations will find, I hope, sufficient justification. I will add a single peculiarity further, which, though not prominent, may not be altogether insignificant.

* Compare for Limax Figs. 43 and 48. See also Whitman's (**'78**[a], pp. 18–21, Fig. 63. *C. P.*) account of it as "the pellucid spot."

† Compare for Limax, Fig. 40; also numerous figures by Bütschli, O. Hertwig, and others.

‡ See Bütschli's figures in Strasburger, *op. cit.*, Taf. VII. Figs. 13, 14.

§ O. Hertwig, **'78**[a], p. 186, Taf. IX. Fig. 12 (wrongly numbered "9").

(3.) The surface of the egg in Strasburger's Fig. 2 is slightly elevated in the region of the questionable corpuscle, somewhat as in Figs. 43 and 50 of Limax.

I am well aware that serious objections to this view of the matter may be raised. The entire absence of anything which could answer to the spindle itself, the thickenings of its fibres, or the deeper sun, is at first thought a weighty objection, and yet I can readily believe that in eggs treated first with alcohol these structural peculiarities may have been obscured by the opacity of the yolk, so that only those parts which lay near the surface were distinguishable. Perhaps a more serious objection exists in the probability that the questionable corpuscles were stained by treatment with osmic acid and Beale's carmine. Strasburger, I believe, does not say directly that such is the case; but even if it was stained, I am not sure that on that account my explanation is to be abandoned. Whitman (**'78**[a], p. 18) says of the "pellucid spot," in the case of Clepsine, that it is deeply colored with carmine, and he too made use of osmic acid. As far as regards this "pellucid spot," I think I have reason to claim that it corresponds with the corpuscle in the centre of the aster of Limax ($a\,a'$), and is not derived from the nuclear plate which Whitman, it is true, did not see, but which could hardly have divided and migrated to the tips of the spindle at so early a stage as is represented by his Fig. 63, or, still less, at the stage of his Fig. 62. So far, then, I have indirect evidence that this flattened corpuscle may stain in osmic acid, and therefore am able to explain its dark appearance in the figures given by Strasburger. I regret that none of my preparations of this stage were made with osmic acid, as, had they been, I might be able to add direct evidence of the staining capacity of these areal corpuscles.

If this explanation be correct, we may confidently expect that the polar globules and their mode of formation will be soon made clear to us in tunicates, and thus one more group of animals be made to lend evidence in support of a rational explanation of the phenomena of maturation which shall be applicable to all the higher animals, if not to all organisms.*

* P. S. — By the last paper of O. Hertwig (**'78**[a], p. 191) my attention has been called to a preliminary notice by Fol (**'77**[h], p. 339), in which he mentions the existence of two polar globules in the case of Phallusia, that I had entirely overlooked. The oversight was due to the incomplete manner in which the contents were indicated on the cover of the magazine in which Fol's article is published. He has *two* articles in the October number of the magazine, but his name appears only *once* on

In his criticism of Hertwig, Strasburger endeavors to show the impossibility of accepting the view that the germinative dot persists. Besides the numerous results of other observers which seem irreconcilable with it, a prime objection is, that it leaves no chance for the existence of polar globules, which Bütschli and Fol have connected in their origin with the germinative vesicle. One has only to assume that the half, and not the whole, of Bütschli's spindle is ejected, the other half remaining in the egg, in order to bring his own (Strasburger's) observations on the "canal cells" of conifers into harmony with the results of Bütschli and Fol. For Strasburger (*loc. cit.*, pp. 293 – 297 and 18 – 21) makes the very important discovery that in conifers the so-called canal cells present in their formation points of resemblance to the polar globules of animal eggs. After the cell nucleus has remained some time at the end of the egg which is to receive the pollen tube, it is divided a short time before the fecundation into halves which are at once separated by a "Hautschichtplatte." One half, which is accompanied by only a very small amount of protoplasm, becomes the nucleus of the canal cell; the other half remains in the egg and in migrating from the pole leaves stretched behind it fibres [interzonal filaments], which in turn disappear while the nucleus, increasing in size, advances to the centre of the egg. This is the "Eikern" (female pronucleus). The formerly expressed idea that the canal cell is a rudimentary structure without recognizable function is to be modified, inasmuch as it is the equivalent of the polar globules, and by its formation the nucleus of the primitive egg (Eianlage) frees itself of certain ingredients, and thus prepares for the approaching fructification. The protoplasm of the canal cell perishes without function. He also finds the canal cell in Cycas. For mosses and vascular cryptogams, however, only the "Bauchkanalzelle" is to be

the cover. Having found the *first* article, the existence of a second, which occurs some pages farther on, was not suspected.

Fol's description is limited to saying that the polar globules arise after the disappearance of the germinative vesicle, and are produced by a process of cell division. As this notice is not accompanied by figures, one is left without the means of definitely confirming or rejecting the opinion I have expressed above about the eggs studied by Strasburger; the mere announcement, however, that polar globules have been seen, only gives greater probability to my explanation.

Since writing the above, I have been able to consult Stossich (**'77**, p. 225), and find that he states, in a criticism of Rabl's theory of the significance of the polar globules, that he has found these directive vesicles "in eggs of serpulas, *ascidians*, and other animals subject to regular segmentation." This paper antedates that of Fol by some three months, but does not contain any description especially devoted to the formation of polar globules in the ascidians.

considered as the equivalent of the canal cell of conifers. The so-called "Fadenapparat" of the egg of angiosperms has also rightly been held in Strasburger's opinion to be homologous with the canal cells.

Strasburger concludes (pp. 304, 305) that a part of the germinative vesicle in the animal egg always remains behind, but that this relic does not correspond to the germinative *dot.* Thus it is more than probable that there is an agreement with corresponding processes in plants, where one half of the divided egg nucleus is eliminated, and the other half is modified in one way or another, and may even become indistinguishable.

Priestley (**'76**) gives a purely objective *résumé* of the papers of Auerbach (**'74**), Strasburger (**'75**), O. Hertwig (**'75**), and Van Beneden (**'75**).

Greeff (**'76**[a], pp. 85–87) takes a position intermediate between Van Beneden and O. Hertwig. He in the main corroborates for Asteracanthion Van Beneden's observations, by saying, that the germinative dot first suffers a conversion into granules, that the vesicle then begins to diminish in size and distinctness, and that finally both *appear* to vanish; and then he concludes by saying, "One cannot positively deny that the germinative spot persists, and, in migrating through the yolk, amœba-like, becomes so indistinct as to be no longer distinguishable." He also reports that eggs of A. rubens carefully guarded from fecundation develop in the normal manner, but considerably slower than fecundated eggs (pp. 83–85).

Selenka (**'76**[a], p. 167) writes of the freshly deposited eggs of Cucumaria, that they no longer possess a nucleus, but exhibit at times "a little drop of protoplasm under the egg capsule, — perhaps the excrement of the egg." This is probably to be considered the polar globule. "In the course of one or a few hours a clear nuclear area (Kernhof) becomes visible in the interior, in the middle of which arise new nuclei, composed of eight to twenty small bodies (Kernkeime, Goette) united in the form of a mulberry." Up to a stage consisting of thirty-two segmentation spheres the same peculiar groups of Kernkeime are met with. Afterwards the nuclei take the form of smooth balls, destitute of enveloping clear areas.

Salensky (**'76**, p. 185, Taf. XIV. Fig. 5) figures an egg of Salpa in which two nuclei [the pronuclei] occupy the opposite poles. I believe Salensky is wrong in holding the presence of these nuclei to be evidence of approaching segmentation.

Zeller (**'76**, pp. 255–260, Taf. XVIII. Figs. 21–31) gives an interesting account of the processes accompanying cell division, and also some

observations on the maturation of the ovum of Polystomum integerrimum. A thickening of the yolk forces the germinative vesicle to one side of the egg, when its section becomes more or less crescentic. The vesicle disappears, leaving behind only a homogeneous light space and faint indications of radiation. The spherical form of the yolk is exchanged for a more flattened one. Two nuclear structures appear near the surface after the egg has resumed its spherical condition, and unite in the middle of the yolk to form a nucleus which soon disappears.

Another case of misinterpretation of the pronuclei similar to Salensky's is that of Barrois ('76, p. 16, Pl. XII. Fig. 2), who says, "Certain eggs [before segmentation] present two nuclei; they are the nuclei of the first two spheres of segmentation."

Ed. van Beneden ('76[d], p. 49) thinks the germs of the infusoriform embryos of Dicyema do not lose their nuclei, as eggs do their germinative vesicles, but that the nucleus divides, and thus gives rise to the nuclei of the first two embryonic cells.

Bobretzky's ('76, pp. 97, 98, 100) observations on the stages embraced under maturation are very limited. At a point on the surface of the freshly laid egg of Nassa mutabilis a small whitish spot is to be seen. Nothing is said about the way the polar globules are formed; but there are two recognizable with each egg soon after the extrusion of the latter, and they are joined to the egg near the centre of the white spot, by a delicate filament. A nucleus is no longer to be found; once, however, when the polar globules were both formed, the nucleus [female pronucleus?] could be distinctly seen immediately under the surface of the egg, but there was no nucleolus; the nucleus was homogeneous, and looked like a vacuole. There is a nucleus-like corpuscle inside the polar globules, which gives to them the appearance of small cells.

Rabl gives an account ('76, pp. 316, 317, Taf. X. Figs. 4–6 *C*), and apparently very accurate figures, of the formation of the second polar globule in Unio, so far, at least, as can be seen on living eggs. More than two polar globules were never observed, nor was the second ever produced by a division of the first. The first is usually somewhat larger than the second. He also figures at the *vegetative* pole a cone-like elevation, which has not entirely disappeared when the second globule is forming. The egg is without a germinative vesicle.

In a lengthy consideration of the significance of the polar globules (pp. 331–338) Rabl combats the notion that their elimination is comparable to an act of defecation; for one would then be compelled, he says, to assume quite different physiological processes for the first

stages of development in cases when no polar globules are formed. He then urges in support of his "protective" theory, — (1.) that, as a rule, the polar globules accompany only the "inequal" method of segmentation; (2.) that the place of their origin is always the animal pole of the germ; and (3.) that the specific gravity of the animal pole is less than that of the opposite pole in cases of inequal segmentation. From all this Rabl concludes that the polar globules serve the purpose of elastic balls in preventing the dangerous pressure of the germ against the membrane of the egg. For the ascidians with their primordial segmentation, the pressure being uniform on all sides, not a few, but a large number, of these elastic balls (Testazellen) are provided.

However ingenious this theory may at first sight appear, it cannot claim to be based upon satisfactory grounds, and I am the more surprised that Rabl should have promulgated it in connection with his previous paper on pulmonate mollusks, since in that case such a relatively enormous distance intervenes between the "Eiweisshülle" and the embryo, — a distance so great that one rarely finds the yolk even in the vicinity of the membrane of the albumen until rotation begins, and then, as the author himself admits, this protective function must cease to exist. Apart from the absence of proof that such protection is needed, or is even advantageous to the embryo, or that the polar globules are capable of offering such protection, the links in his chain of argumentation seem to be exceedingly fragile. All authentic observations, it is true, go to show that there exists the constant relation between polar globule and the promorphology of the egg which Rabl has expressed by saying the globule is formed at the animal pole of the germ. That, however, is only the connecting link between two others.

I believe the evidence is still wanting to prove that the animal pole of the egg is specifically the lighter in all cases of inequal segmentation, or, at least, that the difference in specific gravity is sufficient to cause the germ to rest with the animal pole uppermost. My own observations in the case of Limax have not afforded the least ground for such a conclusion. The yolks of eggs left undisturbed for hours have been found to present the same want of uniformity in position which is met with under any other circumstances; individual eggs have been observed for a long time during the early stages of segmentation, the polar globules remaining all the time in such a position as to be seen outside the profile of the yolk. Furthermore, it seems to me, this theory necessitates the assumption that the yolk (or embryo) is specifically lighter than the enveloping medium, otherwise there would be no

pressure of the embryo against the membrane directly above it; but we have not yet the proof that such is universally the case when polar globules exist. The difference in specific gravity will presumably be too little, in most cases, to cause any appreciable pressure in *either* direction along the vertical axis. It is, however, quite another question whether polar globules are cœnogenetic phenomena. Rabl certainly deserves credit for having turned the discussion concerning polar globules in a phylogenetic direction, and, unsatisfactory as his protection theory seems, it does not necessarily follow that there is no ground for his claim that the polar globules are comparatively recent acquisitions. If the globules were limited, as he claims, to eggs with unequal segmentation, there would certainly exist good reason to infer that they were in some way adaptive acquisitions of this latter class of eggs. But the following are a few of the many exceptions which make it improbable that polar globules are cœnogenetic adaptations to unequal cleavage: Hydra (Kleinenberg, **'72**, pp. 46, 47, 51); Lucernaria (Korotneff, **'76**, p. 393); Hippopodius, Sagitta, and Echinoderms (O. Hertwig, **'78**[a]). Indeed, if Strasburger is right in maintaining that the canal cells of conifers, and equivalent cells of both lower and higher plants, are homologous with polar globules, we must apparently go back to a very early point in the history of organisms to discover the origin and true significance of these cells, unless it is assumed they have been *separately* acquired by the two recognized groups of higher organisms.

Rabl has realized the sentiment of Von Baer's with which he closes his last paper: "Irrige, aber bestimmt ausgesprochene allgemeine Resultate haben durch die Berichtigung, die sie veranlassen, und die schärfere Beachtung aller Verhältnisse, zu der sie nöthigen, der Wissenschaft fast immer mehr genützt, als vorsichtiges Zurückhalten in dieser Sphäre."

Stossich (**'76**) entertains views of the morphology of the egg which are at variance with well-established information. He is apparently influenced in his opinion by the study of the germinative vesicle undergoing metamorphosis.

The egg, he says, is a cell, but the nucleus of this cell is not the germinative vesicle; it is the germinative dot, and within it may be found the nucleolus. The body of the cell, i. e. the yolk, is composed of two layers, — an external, adapted to the formation of granules, and an internal (germinative vesicle), homogeneous and hyaline, in which are contained the nucleus and nucleolus. The so-called germinative vesicle is not, in his opinion, a vesicle having a proper membrane,

but really an optical effect produced by the differentiation of the protoplasm.

If it is not the nucleus of the first segmentation sphere which has misled the author into denying the existence of a membrane, it must be that he has only seen the germinative vesicle after the beginning of its metamorphosis. If there were previously any chance for doubt, the peculiar spindle shape which both nucleus and germinative vesicle assume now proves sufficiently the morphological equivalency of the two structures.

Immediately upon contact with the fecundating element in the case of Serpula the granules of the yolk are much increased, and are seen undergoing a slow rotary motion; this causes an opacity of the yolk which renders the internal layer (germinative vesicle) almost invisible. The latter, therefore, does not disappear, but is simply obscured. This formation of granulations is not simply a mechanical alteration, but is accompanied by a chemical process that eliminates from the yolk certain secondary liquid and gaseous products which accumulate between the yolk and its membrane; it is thus that the yolk becomes somewhat contracted and the membrane much dilated. In consequence of the formation of granulations the external layer of the yolk becomes more dense, and therefore the internal layer (germinative vesicle), being less dense, is obliged to ascend to the surface of the yolk, and thus one pole of the egg becomes clearer than the opposite pole. When the vesicle has reached the superior pole of the egg, the vitelline membrane is resorbed and an aperture formed through which the vesicle escapes in the form of two or three drops, — "directive vesicles," — which remain between the membrane and the yolk. These directive vesicles only serve to determine the point of departure and the direction of the first cleavage furrow.*

The latter is not, however, the first indication of segmentation. After the formation of the directive vesicles the rotary motion of the granules ceases, and they are gradually arranged in two groups between which the plane of division is to pass. The granulations do not remain quite fixed, but are disposed in rays which depart from the centre of each group. Gradually this centre enlarges until it acquires the form of a nucleus, so that the rays produced from the granulations no longer depart from a point, but from a circle. As the furrow of segmentation advances, the rays become more uncertain.

* It is an error for Stossich to connect with this view the name of "the distinguished Müller, father of embryology," instead of that of Friedrich Müller.

Stossich seems to have made, with some other observers, the mistake of confounding the astral areas with new nuclei.

Fol ('**76**, pp. 111 – 113, 138 – 145, Pl. IV. Fig. 3) describes, but does not very fully illustrate, the phenomena of maturation in the Heteropods. "The nucleus had already disappeared in all the eggs (Firoloides) which I have observed, to reappear before and after the escape of the corpuscules de rebut." This statement, with some parts of the immediately following description, is certainly unique. Perhaps the account may be the result of a faulty combination of observations.

Fol gives for Pterotrachea the details of the changes, of which the above quotation is an epitome, as follows. The molecular star has the same appearance as in Pteropods. There is, however, this difference, that the protoplasm is so scanty as to form only a thin layer between the nucleus * and the "protolécithe." When the nucleus [germinative vesicle ?] has vanished, the vitellus appears composed merely of two very clearly marked spheres set concentrically one within the other. The sphere within is nothing else than the protoplasm, the greatest part, but not the whole, of which *surrounds itself with a membrane, and becomes a central nucleus.* At opposite (nutritive and formative) poles of this nucleus there soon appear two centres of attraction whence protoplasmic rays emerge in all directions [first archiamphiaster]. The stouter of these striations stretch from one centre to the other in the interior of the nucleus. The limits of the latter disappear and the stars move apart. Bütschli's fusiform body is only the central part of the vanished nucleus. As to the fibres of the spindle, they are only striations in the protoplasm. One of the stars approaches the centre, the other nears one pole of the vitellus and there gives rise to the first polar globule. In the interior of the globule is readily to be distinguished the termination of the spindle fibres (Bütschli), which have their centre at the middle of the exterior extremity of the globule. There enlargements of these striæ are also to be observed. The star in the vitellus now divides anew, without having taken the form of a nucleus. During this division the striations, arranged in the spindle form, reappear. Then the second globule is formed, in the same man-

* It is often difficult to comprehend Fol's meaning because he uses the term "nucleus" in the most general sense, when accuracy demands a more explicit term. Here, for example, he speaks of the existence of a nucleus where, to judge from what has preceded (Firoloides and Pteropoda), one has the right to suppose that the germinative vesicle has been supplanted by a molecular star, and that consequently there is no nucleus.

ner as the first. After the escape of the [second] polar globule, that which remains of the *star* approaches again the centre of the vitellus, and becomes rounded in the form of a nucleus. The nucleus not only disappears before each segmentation, but it *twice* becomes fused with the surrounding protoplasm and twice individualized before the first segmentation.

In this description that which least coincides with the ideas I have formed from my own observations and those of others is the statement made in the last sentence, together with that which makes the nucleus (germinative vesicle) disappear, and again appear before the formation of the polar globule. Although recognizing the spindle fibres as stouter than the remaining rays, Fol does not admit any fundamental difference between the two. The *equatorial* thickenings were not seen, and the *lateral* zones only in the formed polar globule. I have elsewhere stated my reasons for inferring that the changes succeeding the formation of the first polar globule are more complicated than has hitherto been assumed by Fol or any other observer.

I wish to call attention to only one or two points in his critical review of other authors and in his "reflexions." Touching the *rôle* of the nucleus, Fol says: "It cannot serve as a centre of attraction presiding over cellular division, since these centres of attraction arise at the very limit of nucleus and protoplasm, and since the nucleus, if so be that it is able to persist and divide, would undergo these successive modifications only in a manner altogether *passive*, at least as passive as the cell or the segmentation sphere in which it is situated." And again, "*Il ne se divise pas, il est divisé.*" I believe there are very good grounds for adopting this opinion.

The fibres of Bütschli are filaments of sarcode, according to Fol, and the grains (thickenings) are varicosities of the filaments, which have nothing whatever to do with the nucleoli. This is the first paper, I believe, in which Fol admits that the nucleus does not appear to be dissolved. It changes in volume and appearance, and loses its contour, he says, and its substance obeys the call of the centres of attraction, which, so to speak, tear it in two.

Bütschli ('**76**, pp. 215 – 249, 380 – 394, Taf. I. – IV.) contributes valuable information on the features of maturation in eggs of worms and gasteropods, a part of which was made known in his preliminary account. In Nephelis the youngest eggs studied exhibit near the somewhat flattened pole the spindle-shaped body already described, lying with its axis nearly in the axis of the egg. A broad equatorial zone of thickenings

occupies the middle of the spindle. Around each of its ends is a clear homogeneous area, and outside this the yolk granules are arranged in radial lines, thus forming two suns. The "area" passes gradually into the surrounding granular yolk. The spindle is the metamorphosed germinative vesicle. It is ejected * from the yolk, not in the simple form previously described for Cucullanus, but in several vesicular portions which apparently enlarge by swelling up, and are united to each other by narrow necks. The constrictions are due to an active process of *nuclear* division. Of the three portions of this ejected nucleus (now a polar globule) the first is the smallest, the last the largest. During the early stages of this elimination the part within the yolk retains its spindle form, and a zone of dark granules is found at [near] this end of the spindle, as well as in the part of the polar globule already eliminated. The latter zone is joined by delicate filaments with the filaments within the yolk. Ultimately the whole of the spindle is eliminated. The first and second portions of the polar globules subsequently unite, and a clear vesicle [nucleus] often makes its appearance therein.

The female pronucleus was observed at an early stage, but no connection with the spindle was detected. The changes in Cucullanus have already (p. 404) been given. I will add, that Bütschli (*loc. cit.*, p. 224) judges, from the appearance of the optical cross-section of the spindle, that the nuclear plate lies within a definitely circumscribed body, therefore cannot be a simple differentiation in the yolk. The protoplasm immediately underlying the ejected spindle is for a certain distance clearer and more coarsely granular than the rest of the yolk, from which it is quite sharply defined. This clear protoplasm probably spreads over the surface of the vitellus and is the seat of the formation of the new nuclei, since the latter arise close under the surface at widely separated places.

* I am not quite satisfied what share Bütschli intends to ascribe to the astral rays in this process of elimination. He says (p. 216): "Etwas spätere Stadien zeigen nun, dass die um das eine Ende des spindelförmigen Körpers befindliche Dotterstrahlung bis in die Oberfläche des Dotters gerückt ist und der spindelförmig metamorphosirte Kern sich durch diese Strahlung aus der Oberfläche des Dotters hervorzuschieben beginnt." I think he has nowhere else intimated that the rays were an *agent* in the propulsion of a nuclear mass, and it is therefore barely possible that here he only means to say that the nucleus makes its way *through* (hindurch) the peripheral aster. But the more literal reading makes the rays the agent of the ejection. It is in this sense that O. Hertwig (**'77**, p. 5) understands the author when he paraphrases his description by saying: "Die Kernspindel . . . . wird *von* einer hier befindlichen Dotterstrahlung aus der Oberfläche des Dotters hervorgeschoben."

The least intelligible part of the observations on Cucullanus is the total ejection of the spindle in an *undivided* state, and its resting intact on the surface of the yolk. This is less likely to be a normal condition of affairs from the fact that stages in the formation of the polar globule quite like those observed in other animals were seen and figured by Bütschli.

Observations on several non-parasitic nematodes contribute nothing of special interest concerning the formation of the polar globules, but confirm the existence of two pronuclei which ultimately become fused.

The observations on Lymnæus and Succinea confirm the conclusions reached with Nephelis. The earliest eggs studied exhibit a flattened yolk; one of the poles of the axis thus determined is characterized by a low, broad, conical elevation of clear protoplasm. In the short axis lie two "areas" and their suns, one near the centre, the other nearer the elevated pole, and between the two stretch curved fibres (spindle body). Zones of thickenings do not seem to have been observed till the polar globule was already formed, at least none are figured. This spindle figure migrates toward the surface till one of the "areas" comes to lie in the surface of the elevated portion of the yolk. This area does not seem to have exhibited any central flattened corpuscle such as Limax shows; but aside from this and the absence of lateral zones the stage represented in Fig. 2, Taf. IV. of Bütschli's work corresponds very closely with Fig. 50 of Limax. The polar globule is produced as in Nephelis, but the author thinks he has seen the *already formed second* globule lying still within the yolk, and joined to the globule already ejected by a slender pedicel, — an observation that one cannot now expect to see confirmed. There is some confusion in his mind regarding the persistence of these two systems of rays, apparently resulting from an incomplete conception of the relation of the polar globules to the spindle. The protoplasm found at this pole immediately after the ejection of the first polar globule is limited by a sharp but feebly expressed boundary from the granular protoplasm of the yolk. I have never seen it so sharply defined as he portrays it in his Figs. 3, 5, and 17, Taf. IV. It was this feature which helped to mislead the author into the earlier belief that a remnant of the germinative vesicle remained behind in a recognizable form.

As to the formation of a new nucleus Bütschli says that close under the surface where the polar globules are formed there appear in Lymnæus a number (nine or more) of small nuclei close together. Each possesses a distinct dark membrane, and, within the clear fluid contents, a few dark corpuscles with clear centres. The latter adhere closely to the membrane. These nuclei melt together, so that at a later stage, although

retaining the same structure, they are fewer and larger. As they increase in size, the corpuscles increase in number. At length there are only two large nuclei, and these finally become united. In Succinea there are *never* more than two, and they may arise far apart.

Studies on Rotifera and the pseudova of Aphidæ only resulted in showing that no elimination took place, although the germinative vesicle underwent regressive changes and became indistinguishable.

On the strength of his own observations upon the fate of the germinative vesicle, Bütschli comes to the conclusion that it is possible to explain divergences of opinion, and, in particular, that the steps supposed to be preparatory to division are referable to the formation of a spindle and stellar figures, but that this apparent preparation for division never leads to that definite end, inasmuch as a process of expulsion supervenes. His belief that *only* the vesicle was expelled, and that the *whole* of the spindle body suffered this fate, compelled Bütschli to assume that, on the one hand, the extruded mass in certain cases (Nephelis) increased in volume by a process of swelling, and that, on the other hand, the germinative vesicle might suffer a reduction in size by the loss of fluid constituents during its conversion into a spindle. Thus were the differences in volume between vesicle, spindle body, and polar globules to be explained. It is now certainly established by his observations, he thinks, that the Richtungsbläschen in snails, nematodes, and leeches represent the ejected germinative vesicle, and most likely the whole of it, since none of his observations indicate that any remnant of the vesicle is left behind save the fluid elements which escape at the time of its metamorphosis into a spindle.

Bütschli thinks that the structure held by Oellacher to be the radially striate membrane of the germinative vesicle in the trout should not be interpreted in that way; on the contrary, it is a modified portion of the yolk, and is to be considered as the equivalent of the radial striations which have been observed by himself and Fol. Such being the case, the real extrusion of the germinative vesicle occurs later than Oellacher maintains, viz. only *after* fecundation. Numerous other special cases, which appear to controvert his ideas of the connection of fertilization with the extrusion of the vesicle, he thinks can be explained by the fact that the vesicle *seems* to disappear, but really assumes the spindle condition, and is not actually eliminated before fecundation.

In an appendix devoted to a refutation of O. Hertwig's idea that the germinative *dot* persists as the "Eikern," Bütschli (pp. 432–437) expresses the opinion that this "egg nucleus" may in the case of Toxopneustes represent the whole germinative vesicle, reduced in size after

the disappearance of the *dot*, or at least a part of it; but in general one must conclude from his own observations, as well as those of other observers (Strasburger, Flemming), that the nucleus, metamorphosed into a spindle, is ejected from the yolk. From his own studies he finds no occasion for assuming that this ejection is incomplete. Still, in view of the positive evidence that in conifers a portion of the egg nucleus, as shown by Strasburger, remains behind, he is compelled to admit that he cannot with absolute certainty deny that a part of the nuclear plate of the metamorphosed egg nucleus becomes detached during the elimination to form the basis of one or several of the little nuclei which afterwards appear in the yolk. Two points in his own observations may also be favorable to this view: the origin of the new nuclei in definite spatial relation to the place where the nucleus is ejected, and the apparent connection (in Nephelis) of the eliminated egg nucleus with some of the newly formed nuclei by means of fine filaments.

The signification of the polar globules as understood by Bütschli will be further considered in connection with the subject of fecundation.

Korotneff (**'76**, pp. 392–394, Pl. XVI. Figs. 10–13) reports for Lucernaria the existence of a *micropyle*, which is quite readily seen in fresh eggs. He says it is placed in a depression. It appears as a round clear spot (Fig. 12) when seen from above. The germinative vesicle at the maturity of the egg moves from the interior to the surface. At the same time it takes on an elliptical form and *its peripheral extremity appears to become fused with the vitelline membrane.* "*It has appeared to me*," adds Korotneff, "*that the micropyle is always formed at the place of this union.*"

From the latter part of this description, which I have taken the liberty to italicize, I think it is nearly certain that the supposed micropyle is the same as the "pellucid spot" seen by Whitman, — the corpuscle in the central area of the superficial star of the archiamphiaster. It is peculiar, and perhaps an objection to this view, that the surface of the egg is at this point depressed rather than elevated, yet a like peculiarity is to be observed in eggs of Pterotrachea. (O. Hertwig, **'78**^{a}, p. 208, Taf. XI. Fig. 8.) However that may prove to be, I think this explanation will not be found to contravene any of the further observations made by Korotneff, who finds in freshly deposited eggs that a globule *

* Korotneff erroneously holds this polar globule to be the expelled germinative dot, just as Lovén and others have long ago done.

It is only a typographical error, which here (p. 393) makes Lovén responsible for the idea that the nucleus (instead of nucleolus) escapes as polar globule.

occupies this depression in the surface of the yolk, when only a trace of the germinative vesicle is to be seen (*loc. cit.*, Fig. 13). The failure to make use of proper reagents is sufficient to explain the absence of every thing relating to the internal appearance of the egg at this time.

SCHENK (**'77**) claims for the germinative dot of Serpula nearly the same function which was ascribed by F. Müller to the "Richtungsbläschen." On a former occasion he did not find polar globules, but instead a flattened body which became pressed into the yolk and ceased to be visible. He now maintains that this structure can be considered the expelled germinative dot, for after fecundation one can follow it from its existence within the germinative vesicle until its complete emergence from the egg. After its exit, it is at first round, and only later becomes flat; or on the same egg there may be an *alternation* of these forms. The appearance of this corpuscle is followed by the well-known retraction of the vitellus and the appearance of a radial striation in the protoplasm of the egg. This eliminated dot exercises in part the function of polar globule; it exerts a mechanical or other influence over the yolk which leads to the production of furrows,—an impulse to cleavage. It is difficult to say whether the dot communicates this impulse through some "Impression in den Dotter," or whether some other stimulus is present.

The grounds urged for this opinion do not appear to me of great moment. The elimination of the corpuscle at the point of the surface where the first furrow is soon to appear, and the existence of the corpuscle in the furrow when the latter does appear, are sufficient to show that accurate observations will make of this corpuscle a polar globule, but not sufficient to give support to the theory here propounded.

In a note on fecundation FOL (**'77**) takes the position that there are two well-marked cases in early stages of development: in one, which is exemplified by the sea-urchin, there is a complete absence of the "corpuscule de rebut," the ovule at the moment of extrusion being already destitute of germinative vesicle and possessing only a female pronucleus; in the other case, embracing most other animals, the vesicle is replaced by a double stellate figure, one of the stars escapes to form the first polar globule, and the second polar globule may be formed by a division of the first, or, more often, like the first, by the formation of a second double star. The substance thus expelled corresponds to the major part of the germinative vesicle enveloped by a little vitelline protoplasm. The principal difference in these two cases consists in the epoch of the disappearance of the germinative vesicle, whether precocious or tardy.

In a subsequent note Fol ('77″) gives the results of recent studies on Asterias glacialis. When the ovule comes into the sea-water the germinative vesicle shrivels and in some way melts in the vitellus; its contents do not escape, as Van Beneden thought. The germinative spot also loses its sharp contour, becomes pale, often changes form, continues to diminish, and finally dissolves. Then there remain in the yolk only two ill-defined spots, one where the vesicle was located, the other, of ovoid form, approaches the surface. The use of reagents discloses the existence of a double star, which Fol names *amphiaster*. In its neutral plane this amphiaster often presents bodies of an irregular form which he considers as the remnant of the *membrane of the germinative vesicle.* This is, I believe, the first time Fol records his observation of anything answering to an equatorial nuclear disk.* The remnant of the dot is still visible at some distance from this "amphiaster de rebut," but the author "dares not affirm that no fragment of the germinative dot can enter into the composition of this amphiaster." He afterwards, however, asserts that the *female pronucleus has no genetic connection with the nucleolus of the ovule.* Fol thinks this first stellate figure (*amphiaster*) is not yet that which gives rise to the "corpuscules de rebut," but that it divides within the yolk in such a manner that its peripheral star alone gives rise to the *amphiaster* which is to be expelled. Thus, it is evident according to his description, that there must at one time be at least *three* stellate figures in the yolk. May he not have mistaken the star of a male pronucleus for one of these three? At least I see no other explanation of this statement, for it is quite improbable that any such division as he indicates really takes place in the first amphiaster.

The internal half of the "amphiaster de rebut" doubles, and the second globule is formed like the first; the internal half of this amphiaster changes into a small spot, and becomes a female pronucleus, which migrates toward, but does not reach, the centre of the yolk. These changes are all effected in the same manner, whether fecundation has preceded or not; if fecundation does not now follow, the egg gradually decomposes. It was never seen to develop parthenogenetically.

O. Hertwig ('77) arrives at important conclusions from studies communicated in his second paper on "Bildung, Befruchtung und Theilung des thierischen Eies." The observations were made on eggs of Hirudinea and Rana. To the investigation of the former Hertwig was led by the researches of Bütschli, and undertook their study with the pur-

* Consult in this connection pp. 429, 430.

pose of answering three questions which were left by Bütschli in an unsatisfactory condition: (1.) how the germinative vesicle transforms itself into the nuclear spindle; (2.) whether the fecundation is of influence on the origin of the nuclear spindle and the polar globules; and (3.) whether the nucleus (germinative vesicle) is completely eliminated, or is partly retained and passes into the segmentation nuclei.

The ovarian eggs of Hæmopis contain a comparatively small germinative vesicle with membrane in which are found a single nucleolus and accessory nucleoli; the latter are stained deeply in osmic acid and carmine. For this reason they are both to be considered nuclear substance. At maturity the nucleolus divides, and the nuclear membrane dissolves so that there remains in the egg only a clear spot destitute of granules in which parts of the stained nucleolus are observable. One finds in place of this a spindle, which is variously situated, either near the centre of the yolk, or more to one side, and then often with its axis radially placed, one end being at the surface. When centrally located it is surrounded by the same clear area which surrounds the fragments of the nucleolus at an earlier stage. The fibres of the spindle converge in two points, which are sharply expressed in Hertwig's drawings; they are formed, he says, of compacted nuclear substance, which takes the form of a single dark nucleus (Kern) or several such. This is surrounded by a clear area of protoplasm, around which the yolk granules have a radial arrangement. The middle zone of thickenings becomes especially prominent when the egg is treated with reagents. Hertwig considers as stages in the process of the formation of the spindle, (1.) a condition in which there are in the middle of the egg two homogeneous areas close together, around which the yolk granules are arranged in rays, — there being between these two systems a number of dark, coagulated, irregularly formed corpuscles, which have the appearance of nuclear substance; and (2.) a condition in which there is found in place of these corpuscles an indistinctly limited structure of approximately spindle shape, in the middle of which are found small condensed granules not yet arranged into a regular granular disk.

From these observations he finds reason to dissent from Bütschli's view that the spindle is the entire germinative vesicle metamorphosed. He disagrees because of the great difference in the size of these two forms of the nucleus; the absence of a distinct membrane about the spindle; and the condition of the egg, so often met with, in which the germinative dot or its fragments were the only parts of the vesicle that were preserved. Hertwig does not deny a genetic connection between the

two forms of nucleus; on the contrary, this connection is supported by the following facts: —

1. They both occupy the same position in the yolk.

2. The clear non-granular area which surrounds the centrally located spindle corresponds very nearly with the size of the germinative vesicle and appears to result from its dissolution.

3. An enuclear condition of the egg, if properly treated, could never be made out.

4. The evidences of the dissolution of the germinative vesicle and the formation of a spindle can be arranged in a continuous series.

"At maturity of the egg," — thus Hertwig summarizes, — "the germinative vesicle undergoes a series of changes in that its dot breaks up into several pieces, its membrane is dissolved, and the nuclear fluid (Kernsaft) mixes in part with the yolk. These changes are to a certain extent independent of each other, inasmuch as the dot may persist when the membrane is already dissolved, and *vice versa*. *Out of the fragments of the nucleolus and the remnant of the nuclear fluid arises the fibrous spindle-shaped nucleus.*" Whether the accessory nucleoli, and whether the whole or only a part of the nucleolus, contribute to this spindle is uncertain. The migration to the periphery of the vitellus may take place in either of the two conditions of the nucleus.

The further changes which the excluded egg undergoes within the cocoon were traced on another genus, — Nephelis. The spindle lies already at the time of exclusion in a radial position, with one end near the surface. The first changes are as follows: the rods of the middle zone (Kernplatte) elongate; the homogeneous areas, especially the peripheral, become larger, and the surrounding rays more prominent and extensive; the peripheral area is visible in the living egg. Passing over so much as relates to what may be seen on the living egg of the formation of the first polar globules, I will enumerate only the internal changes. With the formation of a protuberance of the protoplasm at the animal pole the spindle moves farther and farther from the centre of the egg, for its peripheral tip remains as if attached to the summit of the elevation. The middle zone of thickenings splits into halves, which migrate as in nuclear division generally. The granules remain united by nuclear filaments. In consequence of this, the spindle has increased considerably in length.* It therefore comes to lie, when the pinching

* It seems to me that the lengthening of the spindle is very inconsiderable till near the close of the constriction which forms the polar globule, so that it is not quite exact for Hertwig to refer the lengthening of the spindle to any of the preceding

off of the conical protuberance to form the polar globule begins, half in the latter and half in the superficial layer of the yolk. The radiation has meantime diminished, especially in the polar globule, where there is to be seen only a very indistinct arrangement of the protoplasm around a dark granule, the peripheral apex of the spindle.

I can only confirm for Limax this description, which agrees in every essential particular with what I have seen. In one point, however, I have been less successful than Hertwig. I have not seen the continuation of the spindle fibres to the *centre* of the clear astral area. I observe, moreover, that Hertwig has not uniformly represented the apex of the spindle as occupying the centre of this astral area (e. g. the deep end of the spindle, *loc. cit.*, Taf. II. Fig. 2).

Of the lateral zones of thickenings Hertwig adds that they appear, when viewed lengthwise of the spindle, as two circles (not rings) of shining granules. About two hours intervene in Nephelis between the corresponding stages in the formation of the two polar globules. The changes transpiring during this interval, as I have elsewhere indicated, have hitherto eluded most, if not all observers.*

This hiatus in his observations was recognized by Hertwig, for he says (p. 27) this point — the formation of the second spindle — has remained obscure. According to the ordinary course of nuclear division the half of the spindle which remains in the yolk should at first be converted into a homogeneous nucleus, and only then elongate. Some of his preparations also seem to favor the justice of this conclusion; namely, those where the granules of the semi-spindle remaining in the egg had imbibed nuclear fluid and formed small vacuoles. As other intermediate stages were wanting, Hertwig did not feel able to deny the possibility of a completion of the spindle in a more direct manner.†

phenomena, especially not to the separation of the halves of the nuclear disk. This is at once evident, I think, from his own figures, as well as from those I have given of Limax. Compare Fig. 40 with Fig. 43.

* It is true Fol (**'77**[c], p. 448) allows the second spindle to arise by a simple lengthening of the half of the spindle fibres remaining in the yolk, and an elongation of the fibre thickenings; but this conclusion may perhaps not be considered as authoritative and final until it has been shown that intermediate stages cannot have been overlooked. Figures of such intermediate steps as will be a certain guaranty against mistake have not, I believe, been published. There is the more reason for not giving his conclusions too great prominence in this matter, since he affixes so little importance to the spindle fibres, and has in the work just cited figured for the first time in his writings their *thickenings*.

† It may not be quite irrelevant to notice that the intervals which here elapse between the formation of the two polar globules on the one hand, and between the

After the formation of the second polar globule, which somewhat exceeds the first in size, but is otherwise like, and formed like, the first, the half of the spindle remaining in the egg contains a disk of granules, and about its tip a homogeneous area and faint radial striations. A little later a cluster of vacuoles closely pressed together has taken the place of the granules of the disk. These vacuoles are sharply limited from the yolk by a dark lustrous rind having the appearance of nuclear substance, and in the contained fluid small dark granules are suspended. The vacuoles soon increase in size, and flow together into a simple, lobed body, — a nucleus. This female pronucleus migrates toward the centre of the egg, where it meets the male pronucleus. Meanwhile there have appeared in the last-formed polar globule numerous vacuoles in place of the granular zone which occupied its middle. These enlarge and unite into a single vacuole with a dark cortical layer, which stains in carmine. The first-formed globule is partially constricted into two. All three remain attached to each other, and, through the largest one, to the yolk, till about the time of the first cleavage, when they are all combined into a single flattened structure containing three bodies that stain readily. The formation of each polar globule takes place in the manner of a cell division, or, in view of the difference in size of the products, as a cell budding.

Hertwig's studies on Rana are mostly confirmatory of the results reached by Van Bambeke. In the ovarian egg at the time the germinative vesicle is growing most rapidly it presents a spherical form and complicated structure. There is a membrane and about a hundred nucleoli, which are in contact with its inner surface,* and a rich network of finer or broader bands of protoplasmic substance, whose function it is to nourish the nucleoli. The latter are most important components of the nucleus. Already at the beginning of winter the germinative vesicle is found more or less displaced from the centre toward the pigmented pole of the egg, and, although a shrinking in the vesicle takes place, the cavity found outside the vesicle in eggs hardened in

formation of the second polar globule and the first segmentation on the other hand, are very nearly the same, so that the production of a "homogeneous nucleus" and its conversion into a second spindle cannot be excluded on account of any lack of time for the metamorphosis, provided the changes transpire with the same rapidity as they do in the preparation for the first cleavage.

* The nucleoli differ in chemical behavior from the nuclear membrane, with which they do not become fused. In Hertwig's opinion, therefore, Van Beneden's view that both are unaltered remnants of the primitive nucleus (i. e. "nuclear essence") is not tenable.

alcohol is an artificial condition, as Bambeke maintains. The shrivelling of the vesicle is accompanied by a centripetal migration of the nucleoli. Further changes take place only in the early spring. The vesicle then approaches close to the dark pole, and ultimately exchanges its much lobed and folded outline for that of a flattened curved disk. A pigment zone surrounds this disk, — in R. temporaria even on the superficial aspect, — and is continuous with a pigment stripe extending a short way toward the centre of the yolk. The deep end of this stripe is swollen, and embraces a circular clear space connected with a funnel-shaped similar space immediately under the germinative vesicle. No nuclear structure is to be found in the circular spot. The whole results from the closing together of the pigment zone which surrounded the vesicle when the latter migrated toward the surface, and therefore indicates the course it had taken. The method of the ultimate disappearance of the vesicle, which probably takes place about the time the eggs are set free in the abdominal cavity, was not discovered. All the eggs from the body-cavity and the oviduct exhibit the same condition, — the peculiar distribution of pigment matter named by Bambeke *claviform figure*, and the hemispherical clear mass of yolk at the peripheral end of the latter, but not the least thing, within or without the yolk, that could be considered as a remnant of the germinative vesicle. The vesicle is not eliminated in the Amphibia, as in the trout, but is dissolved without recognizable remnant, and mingled with the yolk before fecundation. This takes place, however, only after the vesicle has reached the surface.

Finally, Hertwig discusses at some length (pp. 68 – 71) the signification of the polar globules. The three principal sources of confusion in their interpretation have been: (1.) an exaggerated estimate of the frequency of their occurrence; (2.) a mistaken identification of widely different structures, in that every formed particle of protoplasm between yolk and egg membrane has been considered polar globule; and (3.) the assumption of a genetic connection between two often contemporaneous phenomena, — the disappearance of the germinative vesicle in the mature egg, and the appearance of formed bodies outside the yolk. Since it has been shown that the regressive changes of the germinative vesicle and the metamorphosis of the germinative dot into a spindle-shaped nucleus take place in the ovary a long time before the exclusion of the eggs, and that it is only *after* this that the formation of polar globules takes place, it is evident that the processes stand in no relationship; they must be separately estimated.

While the phenomena of fecundation are, with slight modifications, the same in all cases studied, the process of maturation is subject, in his opinion, to greater variation. The simplest method of producing an "egg nucleus" from the germinative vesicle is by a uniform distribution of nuclear substance in the nuclear fluid, and then by a solution of the nuclear membrane, such as appears to take place in conifers according to Strasburger. With animal eggs, however, the process is more complicated, and there are three methods to consider, of which the simplest is that furnished by Toxopneustes, where the germinative dot persists as "egg nucleus." With the leeches this is modified by the intercalation of the accessory process of forming polar globules, whereby the nucleolus, instead of becoming a homogeneous "Eikern," forms at first a spindle-shaped "Eikern," and only indirectly the homogeneous nucleus. With the amphibians, finally, only a small portion of the nuclear substance — perhaps a single nucleolus — furnishes the diminutive nuclear structure. The last is a modification in the process induced by the *multi*-nucleolar condition of the germinative vesicle.

By studies on Ascaris nigrovenosa Brandt (**'77**) endeavors to refer all the differences in the appearances presented by the germinative vesicle, its supposed disappearance among others, to an amœboid nature, which induces constant change of form. This Brandt claims can be directly observed.

Much of the value which might otherwise attach to his observations is lost from his not having supplemented his work with the proper use of reagents, and from his ignoring the advantages of compression already employed with such success by Auerbach. Brandt goes so far as to express the opinion, that the substance of the germinative vesicle can flow around and envelop the yolk, and that it can assume dendritic forms, become diffuse, disappear, and again collect itself.

With regard to the nuclei [pronuclei] discovered by Bütschli and Auerbach, Brandt, although at first incredulous, satisfied himself of their existence; but instead of arising as minute spots or suddenly as clear balls, they at first present, according to him, the appearance of indistinct, diffuse spots of irregular shape, which, with constant amœboid change of form, at length become rounded, and then appear most distinct. They are not, however, bodies *sui generis*, but rather portions of the germinative vesicle that has become parted by amœboid motion, and is thus reconstructing itself. The mutual approach of these pronuclei he explains as being brought about by a change in the position of the pseudopodia; still the yolk may concur in this move-

ment, especially since a mutual approach of the vesicles is also to be observed while they remain quite spherical. A third cause, he believes, is to be sought in a contractile connecting substance stretched between the two vesicles, in the form of a protoplasmic network, since it is questionable if the contractility of the yolk can effect a regular approach of the vesicles (pp. 371, 379). Finally the latter are completely fused. The germinative vesicle in the egg of the nematode is neither dissolved nor otherwise destroyed.

GIARD ('77) gives a description of hyaline spheres which in Rhizostoma make their appearance near the surface of the egg, and at its maturity constitute a clear zone just underneath the vitelline membrane. As previously (p. 332) stated, he ascribes to Bütschli the discovery that the polar globules are formed in many animals by a process of *cell division*. With all these animals the excreted corpuscles have the value of *rudimentary cells* of an atavistic signification,* and cannot be properly called "corpuscules de rebut." The latter name is only appropriate for non-cellular material rejected by the vitellus which serves for the formation of accessory organs, the vitelline membrane, for example. It is with the latter that the hyaline vesicles in Rhizostoma are to be classed.

The results reached by GIARD ('77[a]) in studies principally on the eggs of Psammechinus miliaris confirm in many points the observations of Fol; in others, his conclusions are different. I shall notice especially their disagreements. The egg of this sea-urchin possesses a very delicate vitelline membrane even *before* fecundation. A little before maturity the germinative vesicle presents the reticulum characteristic of old nuclei. The nucleolus embraces an irregular nucleolinus. The contents of the vesicle become mingled in an amœboid mass, attain the surface of the yolk, and there are converted into a karyolytic figure. The aster directed toward the centre of the egg very rapidly assumes the form of a rounded nucleus, — the structure O. Hertwig took to be the germinative spot. It cannot be the "spot," for it always appears a little smaller than the latter, and moreover one often encounters eggs in which this Wagnerian spot is no longer visible, and in which the female pronucleus does not yet present a distinct nuclear aspect. On the other hand, it is inexact to say that there is no genetic connection between the two (Fol), since the substance of the nucleolus, mingled with that of the germinative vesicle, serves for the formation of the first amphiaster, which gives rise to the female pronucleus. Giard describes the formation of *two* polar

* See also Giard '76.

globules in non-fecundated eggs soon *after their exclusion* (less accurately to be traced before exclusion). In the living eggs one sees two elevations (cumuli) of clear protoplasm, often, though not always, at diametrically opposite points of the surface of the yolk. One arises at the expense of the aster which is fellow to that from which arises the female pronucleus. *This aster* forms an inequal karyolytic figure, of which the small aster becomes the cumulus which produces the first polar globule; the second arises subsequently; both are very small, and disappear quickly. In using staining reagents one finds *two* nuclei at this pole of the egg. The more superficial is the one which by dividing forms the polar globules; the other is the female pronucleus. This method of the formation of polar globules is, so far as I know, quite unique.

The results published by FOL (**'77**[c]) in his paper "Sur le Commencement de l'Hénogénie chez divers Animaux," have been in part given already in the reviews of his preliminary notes. When he says (p. 441) that the internal half of the first "amphiaster de rebut" remaining in the yolk becomes a *complete* amphiaster, one might possibly be inclined to infer from the statement that there was some evidence of the conversion of the internal half of the "nuclear plate" into a veritable nucleus as one of the steps in the process of the formation of the second archiamphiaster. This view, however, is entirely unsupported by what follows. In fact Fol seems to leave no chance for the possibility of such an event, for he says distinctly in this paper (p. 448): "Then the interior aster is converted into an amphiaster in the following manner. Bütschli's filaments, instead of retiring toward the centre of the aster, elongate anew, and the varicosities disappear by being drawn out. These filaments again constitute a spindle (Fig. 7), one extremity of which is found at the centre of the internal aster, while the other point of convergence for the filaments corresponds to the point of contact of vitellus and first polar globule. In the middle of these filaments new varicosities are formed." There is nothing in the figure cited, nor in any other of those given by Fol, which fully warrants the name *amphi*aster, since no trace of a radial influence at the outer pole of the second spindle, save the spindle fibres, is visible, to say nothing of a *complete* aster at this point. A complete spindle is present; a complete amphiaster is not.

On another point Fol gives (p. 447) somewhat more extensive information than hitherto. He still insists that with the starfish the first amphiaster does *not* give rise directly to the polar corpuscles. "If," he says, "one treats an egg with reagents a few minutes after the first amphiaster is formed, one no longer finds an amphiaster, but a compact

body with stellate contour. Does this body correspond to the whole amphiaster, or to only one of its halves? Does it result from a condensation or from a division of the amphiaster? The second supposition would appear *a priori* the more probable;* but as I have never succeeded in seeing at the side of this stellate body another aster, I prefer to adhere [?] to the first supposition." My criticism of the assumption first suggested by Fol — a *division* of the first *amphi*aster — is perhaps intelligible in the light of his first description. With *this* statement of facts, it no longer serves as an explanation. I am, nevertheless, still unable to accept the conclusions which Fol has reached on this point, and believe that the phenomena are to be otherwise explained than by assuming that either a division or a temporary consolidation of the first amphiaster normally takes place. Without personal experience with the animal under consideration it is fruitless to attempt any explanation. Possibly Fol may have been less certain than he supposed of the relative degrees of advancement presented by the stages compared, and that, after all, the unique stellate body may have represented a condition *antecedent* to the first amphiaster, rather than subsequent to its formation. The possibility of such an error is not, in view of the necessary use of reagents, entirely improbable. The failure of other observers to distinguish any corresponding stage in the metamorphosis gives reason to think this may be due to an *abnormal* condition of the eggs in which it was seen.

In living eggs, when the polar globule begins to detach itself, the surface of the yolk forms folds arranged like the rays of a star whose centre is the peduncle uniting the globule to the vitellus. These folds become more prominent as the globule detaches itself, and then fade away. This and other phenomena — the elevation of a distinct pellicle in the formation of the polar globules — the author thinks are easily explained, if one admits that the superficial layer of the yolk has a greater consistence than the yolk itself. Although this layer in certain respects deports itself like a true membrane, in his opinion it is not such.

There result from the internal half of the second archiamphiaster one or two small clear spots, which present, when treated with reagents, the aspect of young nuclei. They increase in size as they sink into the yolk, and become fused together. Other clear spots appear at the side of the first, and they too are fused with it to form the female pronucleus.

Fol also reports the discovery of one (if there are two, the second has escaped observation) polar globule in the sea-urchin. They are elimi-

* It is the opinion previously adopted. See p. 436.

nated while the eggs are still in the ovary, and are formed as in the starfish, with the exceptions as to number and as to their failure to raise any sort of a pellicle. On account of the absence of a pellicle they are soon lost after the exclusion of the egg.

The errors of Van Beneden and O. Hertwig relative to the fate of the germinative vesicle are due, in his opinion, to the use of slight pressure, resulting in abnormal phenomena. Other cases (Sagitta and Phallusia) are cited to show that the vesicle may early disappear.

In Phallusia the "testa cells" arise within very young eggs and in contact with the nucleus, but this is in no way to be compared with the formation of polar globules, so that the sea-urchin is the only animal whose eggs part with their polar globules while still within the ovary.

In Heteropoda after the disappearance of the Wagnerian dot there appear two centres of attraction at the two extremities of the vesicle. The rays of the stars, which announce the existence of these centres, extend partly without and partly within the vesicle. The latter encounter and unite with each other, beginning with those in the middle [axis of spindle?], to form the bipolar filaments of the first amphiaster. After the second polar globule is formed, the varicosities of Bütschli pertaining to the last aster reunite with the central mass of the aster to constitute the female pronucleus. The male only makes its appearance when the second polar globule is forming, notwithstanding fecundation is effected in the oviduct long before. It is at first very small, extremely refringent, and located at the surface of the yolk in a position variable as regards its relation to the polar globules. In the starfish the male aster also remains latent up to the same moment. At a certain stage in the growth of both pronuclei there appears a minute nucleolus. The nucleus of the fecundated egg has only a very remote connection with the germinative vesicle.

The figures given by Brandt (**'77**[b]) to illustrate the formation of the polar globules in Lymnæus cannot be considered as giving a very complete idea of the process. In the author's opinion (p. 591) the globules are formed by a part of the amœboid germinative vesicle swelling forth in the form of a clear rounded drop in which an irregularly outlined nucleus at once appears. It is only a portion of the germinative vesicle which is thus expelled, the most of it returning as an amœboid body into the vitellus, where it becomes indistinct, but still persists, to give origin to the nuclei of the first spheres of segmentation. Brandt's views of the amœboid nature of nuclei are elsewhere discussed.

O. Hertwig (**'77**[a]) gives in a preliminary paper the results of studies

on the eggs of a number of animals made in the early part of the winter of 1876–77, — therefore very nearly contemporaneous with Fol's valuable investigations. As the ultimate illustrated papers (Hertwig, **'78** and **'78**[a]), giving more fully the results on which this preliminary communication is based, have already appeared, I will limit myself here to a statement of Hertwig's general conclusions, and refer the reader for details to the review of those papers which will be found farther on.

Hertwig has also discovered, independently of Fol, the existence of polar globules * in Sphærechinus brevispinosus, which were formed in this case from eggs artificially removed with the ovarium and laid for some time in sea-water. From all his observations Hertwig finds confirmation of his previously expressed views on maturation and fecundation, especially in three points: (1.) that the continuity in the generations of nuclei is not interrupted; (2.) that the polar globules arise by a process of cell budding; and (3.) that fecundation depends on the copulation of *two* nuclei. On the other hand, his opinion in regard to the prevalence of polar globules is altered. He now believes that a general agreement in this matter throughout the animal kingdom will be established. The most important objective communication in this paper is unquestionably the description given of the method in which the first maturation spindle arises in Asteracanthion (see p. 452).

According to P. Mayer (**'77**, p. 199) the germinative vesicle disappears, in the case of Pagurus, while the egg is still in the ovary, so that when freshly deposited it is "positively enuclear." Of this he has convinced himself by crushing the eggs, and has also often observed the origin of a new nucleus. Before it perishes the vesicle is sometimes to be seen near the surface of the egg, — instead of the centre, where it always is at first, — surrounded with its protoplasmic area. This eccentric position he regards as probably abnormal, and indicative of an approaching disintegration of the egg. With the disappearance of the vesicle the protoplasmic area surrounding it ceases to exist. For this reason a direct dispersion of the elements of the vesicle in the protoplasm is the simplest assumption. The protoplasm, retaining its net-like distribution, may subsequently secrete a new nucleus in its centre. Since the existence of a distinct egg membrane (not affected by caustic potash) and of the germinative vesicle appear to exclude each other; and since fecundation must precede the formation of the membrane, he

* The existence of polar globules in the sea-urchins was established by Agassiz in 1867. See A. Agassiz, **'64**, p. 6, Pl. I., **'77**, p. 7, Pl. I., and **'67**[a], p. 2.

concludes that the vesicle disappears *after* fecundation, whether as a *result* of fecundation is uncertain (p. 204). Mayer seems also to have seen in isolated cases of freshly laid eggs a "sort of Richtungsbläschen in process of elimination"; but he considers this process as also abnormal, so that his subsequent suggestion, — that it were, perhaps, not too venturesome to connect this with the eccentric position of the germinative vesicle, — has not that importance in his mind which can fairly be attributed to it to-day.

Stossich ('77) has extended his observations to the Echinoderms, and maintains the same view relative to the morphology of the egg which he previously expressed ('76). The germinative vesicle of the egg mature and ready for fertilization has a perfectly spherical form, but no membrane; its protoplasm is clear, transparent, homogeneous, and slightly granular. He does not know that Hertwig's observations of a delicate network within the germinative vesicle have been confirmed. If it had so complicated a structure, it could no longer be regarded as a cell, but as a much more differentiated organism. The germinative dot always has an eccentric position, is round, and contains a very well pronounced nucleolinus. The author says he has several times had the opportunity of seeing *two* germinative dots in a single egg. They were, however, always joined; in these cases the nucleolini were wanting.

I do not doubt that these two "germinative dots" are really the conjugating pronuclei, although the accompanying figure (*loc. cit.*, Tav. I. Fig. 2) gives no evidence of the existence of polar globules or the elevation of the membrane of the egg at any part of the periphery which is shown.

After fecundation the nucleolinus is no longer visible, and the contours of the dot become always less decided, until they disappear without leaving a trace. The vesicle from being round assumes an irregular dentate outline. This change of form is only the effect of a movement developed within the egg *by reason of contact with the sperm.*

Stossich desires his previous hypothesis, that the germinative vesicle approaches the surface in consequence of the greater density of the external layer of the yolk, to be so far corrected as to grant that this is aided by the amœboid motion of the vesicle. He is unable to say whether the whole of the vesicle escapes as the two or three directive vesicles. After the elimination of the last polar globule the yolk becomes homogeneous, then there is in its centre, after a little time, a round body which becomes more distinct. It is the nucleus of the

first "embryonic sphere," — the analogue of the germinative dot. Its contour at length becomes less distinct and it entirely disappears. With the dissolution of the nucleus the existence of the first embryonic sphere is at an end, although some minutes later there begin to be developed in the yolk certain phenomena which lead to the formation of two new nuclei and to the division of the yolk into two embryonic spheres. The yolk is now homogeneous. Little by little a protoplasmic mass is collected in the centre; this increases and becomes more readily visible, but its contours are blended; the granules cease their rotation and are disposed in rays. The central body becomes elongated in a plane perpendicular to that of the polar globules; the motion is not amœboid; this nuclear body is divided by a constriction, and afterwards the yolk suffers the same fate.

Bütschli (**'77**^{b}, pp. 232 – 237, Taf. XVII.), independently of the recent observations of Hertwig and Fol, radically modified his opinion of the nature of the polar globules. In Neritina fluviatilis he finds that both the fertile and the *in*fertile eggs of a capsule produce polar globules; the former at least three (only one observation), the latter a larger number, sometimes as many as five. It is not possible, he says, to be certain that all the globules are observed, since in opening the capsule they may easily be lost. Staining in Beale's carmine and the subsequent well-known method of decoloration by means of hydrochloric acid furnishes evidence that the polar globules are not composed exclusively of nuclear substance, but that they are each *composed of protoplasm which encloses from one to three small nuclei*; and, further, that the infertile yolk after the formation of the polar globules still embraces from one to three small nuclei, — in other words, that *a part at least of the germinative vesicle remains in the yolk after the production of the polar globules.* Bütschli fully accepts O. Hertwig's view of the origin and nature of the polar globules, but still from a physiological standpoint thinks their principal signification is to be sought in the removal of a portion of the egg nucleus (germinative vesicle), whether this is accomplished directly or under the form of a "Zellknospung."

It is probable, he adds, that the infertile eggs have remained unfecundated. If this be true, Neritina will afford evidence that polar globules may be produced by *un*fecundated eggs, a conclusion which Fol and Hertwig have likewise reached from satisfactory evidence. In view of their extensive prevalence, the polar globules are probably of fundamental significance; their import will receive a sufficient explanation only with a more intimate knowledge of the processes of reproduc-

tion — especially the phenomena of conjugation — among the lower organisms.

A critical review of Bütschli's "Studien," etc., by Dallinger and Drysdale ('**77**), is principally directed to pointing out what is observation and what inference in Bütschli's work.

Hatschek ('**77**[a]), without having devoted especial attention to the phenomena of maturation in Pedicellina echinata, has observed (p. 504) the existence of two or three (?) polar globules of variable size, which are found at the animal pole of that axis which he believes is differentiated in the unsegmented eggs of *all Metazoa* (p. 524). A definitely limited nuclear structure nearer the animal than the vegetative pole (which may be the primary cleavage nucleus) is the centre of a radial arrangement of the yolk elements.

The maturation changes of the eggs of Malacobdella and Clepsine have been incompletely observed by Hoffmann ('**77**, pp. 18–21, and '**77**[a], p. 34). In the former case the germinative vesicle in approaching the surface gradually diminishes in size, but preserves its rounded outline. Two hours after fertilization two polar globules were seen.

With the growth of the egg of Toxopneustes variegatus there appears according to Selenka ('**78** and '**78**[a]) a remarkable differentiation of the cell into three concentric layers. The middle is a very thin pellucid layer of protoplasm without granules, and disappears when the full size is reached. During the later stages of growth, the outer yolk layer sends out pale pseudopodia, which, at first isolated, arise as blunt or bush-like projections of rapidly altering form, but finally assume the shape of very numerous and fine, motionless rays. These, he believes, serve for the growth of the egg. The whole yolk is undergoing change of form during the activity of the pseudopodia. Finally it comes to rest, and the pseudopodia are withdrawn. Meantime the germinative vesicle has suffered changes from its spherical form; its membrane has been variously folded and wrinkled; it has approached the periphery of the yolk after the resorption of the germinative dot. Two polar globules are formed. The place of their formation remains a long time recognizable as an elevation of the surface of the yolk (Dotterhügel). There then appear in the yolk under this elevation several clear bodies which unite to form the "Eikern." The latter moves inward, but does not take a central position in the yolk. It is probable that the "clear bodies" are the product of a budding (Abschnürung) of the germinative vesicle.

The comparison which Strasburger previously ventured to draw between polar globules and the "Bauchkanalzelle" of the higher crypto-

gams and archisperms, makes it of considerable interest to learn the conclusion which he reaches in his more recent studies (Strasburger '**77**) on the "Embryosack" of metasperms. The whole process within the embryo-sac (studied especially in Orchis) is put in a new and unequivocal light. The egg cell, the two "companion cells" (Gehülfinnen), and the "antipodal cells" (Gegenfüsslerinnen) are all formed, not by a free cell-formation, but by the successive divisions of the cell which forms the beginning of the embryo-sac, and with each division the nucleus undergoes a spindle metamorphosis. From these successive divisions there result eight cells, four in each end of the embryo-sac, or more properly speaking eight nuclei, only six of which (three at each end), become definitely circumscribed cells, since the division is in so far incomplete, that one of the four nuclei in each end of the embryo-sac is left free in the protoplasm of the sac not employed to form the six definite cells. The two nuclei thus left free migrate toward each other and fuse (conjugate?) to form a single nucleus. The group of three cells at the posterior end of the sac are the antipodal cells; of the anterior group, two are the "companion cells," whose anterior ends form the "Fadenapparat" when it exists, and the remaining one is the egg cell, whose sister nucleus was the anterior of the two copulating nuclei. The "companion cells" cannot be considered equivalent to "canal cells" (or polar globules), since they are not derived directly from the egg cell. The "free" nucleus is the one last to be separated from the nucleus of the egg cell, but its entirely anomalous fate prevents any comparison with canal cells, or, for that matter, with any other, save copulating sexual cells.

For the present, then, the angiosperms seem to present no opportunity to extend our knowledge of the possible origin of the polar globules. Notwithstanding this there still remain these important facts, to which Strasburger directs attention, since they show that often parts of the cells which are undergoing sexual differentiation are detached at early stages, and are excluded (like polar globules) from the subsequent sexual act: that in Spirogyra Heeriana a vesicular portion of the cell, which at the time of copulation migrates, is excluded from the copulation (in other Spirogyras, however, this is not the case); that in certain algæ, for instance, a part of the egg substance is simply ejected, and also that not *all* of the substance of the antheridium is employed in the formation of the spermatozoids; that in higher cryptogams the "Bauchkanalzelle" is formed, and the spermatozoids carry about for a time a vesicle which represents a part of the "Mutterzelle" and which is in no way con-

nected with the fecundation; and finally, that in the archisperms an equivalent of the "Bauchkanalzelle" is formed, and that perhaps the separation of the contents of a "vegetative cell" in the pollen grains of archisperms has a preparatory significance for the formation of fecundating substance. But a difficulty in the way of this view for the spermatozoids of archisperms is the fact that in Selaginella and other Dichotomeæ *both* the "vegetative cell" and the "vesicle" are present.

Before the maturity of the egg of Asteracanthion, there is, says O. Hertwig ('78), a migration of the germinative vesicle toward the surface of the yolk, where it loses its intra-nuclear network, and where its membrane becomes uneven by reason of infoldings. He recognizes that the germinative dot is composed of two substances, which differ both in the fresh condition and more emphatically when treated with reagents. The smaller portion lies as a protuberance on the larger, or may be entirely surrounded by the latter; it is more promptly and deeply stained, and resists the swelling influence of ammoniacal fluids longer, than the larger portion; the latter becomes in 2 – 4% acetic acid quite transparent, while the former becomes intensely lustrous.

The changes at the time of maturation are inaugurated in the protoplasm which surrounds the vesicle. In the living egg it is seen that a knob (Höcker) of protoplasm pushes its way into the germinative vesicle from the side which lies nearest the surface of the yolk. The apex of the knob embraces a light spot free from yolk granules, and sends out long protoplasmic projections in all directions. The nucleolus now (fifteen to twenty minutes after the eggs are brought from the ovary into sea-water) loses its several vacuoles and thus appears homogeneous; in a short time there arises in its centre a larger single vacuole, that is nearly filled by a solid round body, which by the use of reagents is shown to be the same as the above-described *smaller* portion of the nucleolus. Suddenly this vacuole with its contained corpuscle disappears. What becomes of the corpuscle is shown only by employing reagents. The observed stages probably follow each other in this order: the corpuscle lying in the vacuole elongates, becomes pear-shaped, then club-shaped, at length more rodlike, and finally a series of beadlike enlargements. It has thus come to project with its smaller end through the rind of nucleolar substance surrounding the vacuole, and its extremity is at last found to extend into the protoplasmic knob and to occupy the centre of its stellate figure. This is accomplished in the course of about ten minutes. Then there appear in the centre of the stellate figure granules which consist of nuclear substance and are probably de-

tached from the metamorphosed rodlike body, for the latter ultimately disappears entirely by this process. The granules assume a circular arrangement (I will speak of them as the circle of granules). Hertwig is unable to say positively whether the whole of the other (larger) portion of the nucleolus remains in the germinative vesicle, since many preparations favor the view that particles of *this* portion *now* make their way into the homogeneous spot of the protoplasmic knob. It at least finally disappears, as does also the membrane and later the "Grundsubstanz" of the germinative vesicle.

What becomes of the "circle of granules" Hertwig unfortunately does not state; also the origin of the second stellate figure and the spindle fibres that unite them cannot be considered as satisfactorily explained by these observations.

During the disappearance of the smaller nucleolar body, as seen in living eggs, and soon after the *début* of the first small radial figure, there appears a second like figure *near the first.* In using acetic acid it is seen that there lies between these two stellate figures a fibrous body whose fibres become more distinct as the remnant of the nucleolus disappears. This body ultimately forms the "Richtungsspindel." The latter elongates and takes a radial position, while the asters increase in size.

Just what relation the "circle of granules" sustains to this spindle, I am unable to discover. It is a difficult point that needs to be definitely settled. Perhaps the conclusion nearest at hand is that the *fibres* of the spindle are formed from the *outer* and larger part of the nucleolus; that the *inner* corpuscle of the nucleolus furnishes directly, in the "circle of granules," the equatorial zone of thickenings. But apparently irreconcilable with this supposition is the fact that the "circle of granules" occupies the *centre of the first star*, and that the second star arises *near* (not by a division of) the first.

The more general conclusion,* and one of fundamental importance, which Hertwig reaches in his preliminary paper (**'77**[a], p. 273), seems in the main just, and it is greatly to be regretted that he was not able to

* "Wenn ich die geschilderten Befunde deuten soll, so scheint mir ein unverkennbarer Zusammenhang zwischen dem Auftreten der beiden Strahlensysteme und der Umbildung des Keimflecks der Art zu bestehen, dass bei der Auflösung des Keimbläschens die Kernsubstanz in das Protoplasma überwandert und an dem Orte, wo sie sich zu dem Spindelformig differenzirten Kern ansammelt, erst ein und dann das zweite Strahlensystem hervorruft. In erster Linie ist bei dieser Umlagerung der activen Kerntheile der in der Vacuole des Keimflecks eingeschlossene kuglige Körper betheiligt. Aber auch von der einhüllenden Kernsubstanz gehen offenbar Theile, wenn nicht Alles, in das neue Kerngebilde mit über."

settle at the same time the nature of the share each of these nucleolar structures takes in the formation of the maturation spindle. There is, besides, one important point which is not, even in these studies, made sufficiently clear to satisfy me. I am unable to understand how the substance of the nucleolus is more active in producing the stellate figures than the protoplasm of the yolk. If this radial system is induced by the immigration of nucleolar substance into the protoplasmic knob, then certainly we should not expect the stellate figure *before* such immigration; consequently the question must arise, What is the signification of the clear non-granular spot in the protoplasmic knob? Is it not due to the same agency as that which induces the stellate figure? Is it not, in fact, simply the first trace of such a figure still limited in its extent? But this clear spot *antedates* even that part of the metamorphosis of the nucleolus by which its *several* vacuoles are succeeded by a single larger subcentral vacuole embracing the smaller nucleolar body (compare Hertwig, **'77**[a], p. 271); by so much the more, then, does it antedate the conversion of that smaller nucleolar corpuscle into a rodlike body with its end at the centre of the star. And, further, what shall be said of the "langgestreckte Protoplasmaerhebungen," "which are sent out in the upper wall of the germinative vesicle, raylike, from the apex of the protoplasmic knob on all sides, like mountain ridges from a central peak"? They are represented in Hertwig's Taf. VI. Figs. 2 and 3, at a time when the inner corpuscle is entirely enclosed in the vacuole of the nucleolus, and yet the peculiar radial arrangement of these "Erhebungen" can hardly be due to any other cause than that which induces the stellate figure. If the first indication of the commencing metamorphosis is seen in the invasion of the territory of the germinative vesicle by a protuberance of the surrounding *protoplasm*, what can be the necessity of transferring the initiative activity to the nucleolus, which still preserves its morphological integrity? May it not be that Hertwig, by his commendable exertions in rescuing the nucleus from a position of comparative subordination, has ascribed to this substance undue importance, and given it exclusive control where it is, after all, only one of two co-ordinate factors? A connection there doubtless is between the metamorphosis of the germinative dot and the formation of a nuclear spindle, but it is not so certain that the nuclear substance gives the *first impetus* to the formation of the *stellate figures*, which mark, in some cases at least, the first unequivocal steps toward a spindle metamorphosis. When Hertwig speaks of an "Ueberwanderung" of nuclear substance into the protoplasm, I understand that to imply — as in fact his figures in so precise

and satisfactory a manner indicate — a transmigration of *recognizable morphological fragments* of that substance. If, on the other hand, one were to maintain that dissolved portions of the nuclear substance first escaped the limits of the nucleus (and germinative vesicle), and then were re-collected and thus gave the initiative to the protoplasmic asters, it would be as impossible, with our present means of investigation, to refute as to prove the claim.

A short pause ensues — to return to Hertwig's description — after the formation of the first maturation spindle. The formation of the polar globules follows as in Nephelis. Two points only are of further interest: first, that Hertwig noticed furrows on the surface of the polar globule, as well as of the egg, which converged toward the place of constriction during the budding process, and that the spindle before the formation of the globules becomes broader and shorter.

The possibility of an *indirect* formation of the second maturation spindle, which Hertwig emphasized on a former occasion, neither finds support nor opposition here. The fact that the inner aster has been converted into a "Doppelstrahlung" within a *quarter of an hour* after the formation of the first polar globule, would seem to preclude the possibility of such an event in the case of the starfish. Nevertheless, I think this point is worthy of still further examination.

A zone of granules occupies each of the polar globules; a third, says the author, lies near the surface of the yolk. From the latter is formed the egg nucleus, — just how is not quite evident. In the clear space which these granules occupy there appear later a number of vacuoles, and in the centre of each a granule of nuclear substance. The vacuoles soon become confluent, thus forming the egg nucleus, and later the granules are united into a single structure,* — the nucleolus. The egg nucleus has moved during its formation toward the centre of the egg. Hertwig does not say whether the stellate condition which the protoplasm "nach dem Centrum des Eies zu" has assumed goes in advance of the vacuole or not. It ultimately becomes fainter, and disappears. Hertwig did not succeed in verifying Greeff's observations of the parthenogenetic development of the starfish.

CALBERLA (**'78**, pp. 438 – 447) has ascertained that, accompanying the metamorphosis of the Ammocetes stage into the adult form of Petromyzon Planeri, the germinative vesicle of the ovarian egg undergoes a very slow migration to the surface of the yolk, and a metamorphosis

* In the preliminary paper (**'77**[a], p. 274) it is stated that a single nucleolus arises, after the vacuoles have become confluent, by an "Ausscheidung."

from which an egg nucleus (in Hertwig's sense) arises. The stages of this metamorphosis are not very completely known. Eggs taken between the middle of October and the middle of November from animals approaching maturity exhibit the germinative vesicle, still sharply outlined and already arrived at the periphery of the yolk. Those taken toward the end of November and at the beginning of December, on the contrary, show that the vesicle has already lost its germinative dot and its sharp contour, and only its protoplasm lies in an irregular form at the periphery. Within this mass of protoplasm are observable, in the fresh state of the egg, "all sorts of nuclear structures," which are probably descendants of the germinative dot. In many eggs, however, — and these the largest in the ovary, — there was nothing to be seen of a germinative vesicle or nuclear structures; there was only a clear drop of protoplasm at one point of the periphery. Already, on the 9th of December, the eggs of a completely metamorphosed larva exhibited a new nucleus (Eikern) in this clear mass of protoplasm or remnant of the germinative vesicle. Calberla thinks, without having recorded any direct observations of such an act, that a part of the vesicle is eliminated as the polar globule. The new nucleus then migrates toward the centre of the egg, drawing after it a cord of protoplasm destitute of yolk granules. Thus a month or more before the maturity of the egg one finds the following complications of structure. The egg membrane is thickened and exhibits a micropyle at its narrow end where the germinative vesicle approaches the surface; this he calls an *outer* micropyle, to distinguish it from the entrance to a canal — "Spermagang" — formed directly underneath it in the granular yolk by the centripetal migration of the egg nucleus and the clear protoplasm it carries with it. The *entrance* to this latter canal is the *inner* micropyle. Protoplasm which is destitute of granules envelops the granular yolk on all sides, and is thickened at this, the animal pole, where it is continuous with the likewise clear protoplasm that fills the "Spermagang." Within the enlarged deeper end of the latter the egg nucleus lies surrounded on all sides by a stratum of this clear protoplasm.

Galeb (**'78**[a], pp. 363–366, Pl. XXII. Figs. 1–4) maintains, on much the same ground as the embryologists of ten and twenty years ago, that the germinative vesicle persists, and (without any fibrous metamorphosis) undergoes a simple elongation, constriction, and ultimate division to form the unequal nuclei of the first pair of blastomeres.* He seems to have taken no measures to insure himself against the possibil-

* See also the review at p. 334.

ity of committing the same mistake as the earlier writers, who concluded that the germinative vesicle divided because they saw a nuclear structure (which we now know is *not* the germinative vesicle) undergo such changes as are here reported. The observations of stellate figures on living eggs are too numerous to allow the acceptance of his conclusion that they are due to the use of reagents. The figures given by Galeb are interesting in several particulars. His Fig. 3 (Pl. XXII.) probably shows the pronuclei, which Bütschli figured four or five years ago in a similar situation. Whether it is the germinative vesicle or the female pronucleus which is shown in Fig. 1, it is noticeable that the structure is not in such a position as to warrant the supposition that the polar globules are produced at the equator of the egg, where the first cleavage plane occurs. The position of the globule after its liberation would, of course, be of comparatively little value in determining this point, because of the possibility of its passively being made to occupy a position different from that which it had when first produced; in the case of the female pronucleus or germinative vesicle, however, such a displacement could not be assumed. If future observations directed to settling this point — the mutual relation of the first cleavage plane and the polar globule at the time of its formation — shall show that in some nematodes the globule is formed at the *pole* of the egg, and that the segmentation plane passes through the *equator*, it will be necessary to seek some explanation of this variation from what now seems to be a very general law. The possibility of a rotation of the yolk after the formation of the polar globules, so that the pole of the yolk comes to occupy the equator of its shell, is not to be lost sight of in this connection.

BALFOUR (**'78**[a]), after giving a concise account of recent progress in the study of the maturation of the ovum, states some conclusions which he thinks already warranted by the observations (pp. 120–124). The peculiar changes which the germinative vesicle undergoes at the time of maturation are, in part at least, of a retrogressive character. The budding of the polar cells is entirely independent of impregnation. He says further, "I would suggest that in the formation of the polar cells part of the constituents of the germinal vesicle which are *requisite for its functions as a complete and independent nucleus* * are removed to make room for the supply of the necessary parts to it again by the spermatic nucleus." From the probable absence of polar cells in cases where parthenogenesis is most common, he is led to suggest further, "*that a more*

* The original is not Italicized.

or less essential part of the nucleus is removed in the formation of the polar cells; so that in cases, e. g. Arthropoda and Rotifera, where polar cells are not formed, and an essential part of the nucleus not therefore removed, parthenogenesis can much more easily occur than when polar globules are formed."

"It is possible," Balfour further observes, "that the removal of part of the *protoplasm* of the egg in the formation of the polar cells may be a *secondary* process due to an attractive influence of the nucleus on the cell protoplasm, such as is ordinarily observed in cell division."

Repiachoff (**'78**, p. 412, Figs. 1–10) gives a brief account of the structure of the germinative vesicle and some of the changes which overtake it in the case of Tendra zostericola, but reserves an extended account for a future occasion, when his observations shall have been concluded. In the black, round ovarian eggs the vesicle possesses a distinct membrane; the germinative dot is of irregular form and embraces several irregular vacuoles. When the egg has assumed its peculiar bilateral form the vesicle still retains its membrane, and there is then to be found in stained eggs within the germinative vesicle a single, or sometimes two nucleoli, and other spots less deeply stained than the nucleoli, but more deeply than the nuclear fluid. Sometimes it was impossible to find evidence of the existence of a germinative dot in any form. He only hints at the possible fate of the vesicle, and then calls attention to the existence of two polar bodies ("Excretkörperchen"?) differing considerably in size, which were observed in the plane of, and just prior to, the first segmentation.

The peculiar growth and activity of the egg of Toxopneustes variegatus has already been given. Selenka (**'78**^{a}) adds in the present paper that in the germinative dot there arise vacuoles, which appear to lead to its complete dissolution; of this, however, he is made doubtful by the different results obtained by O. Hertwig. He is in accord with Fol and Hertwig as regards the formation of polar globules by the division of a spindle and the re-formation into the "Eikern" of so much of the latter as remains in the yolk. It is perhaps doubtful if "pronucleus" is in a morphological sense a proper expression, since neither sperm nucleus nor egg nucleus can alone play the *rôle* of a cell nucleus.

While the polar globules emerge, a drop of protoplasm free from granules flows out and soon envelops the whole yolk in the form of a cortical layer endowed with automatic motion. Its fate is threefold: (1.) its outer limiting layer is afterwards elevated as a vitelline membrane; (2.) a part penetrates with the spermatozoön into the "clear

area" of the first vitelline "sun" (though often observed, this is thought by the author to be without significance); but (3.) the greater portion is drawn into the segmentation cavity during the beginning of cleavage, where it helps to form the "Gallertkern." The "Dotterhügel" remains, and with some exceptions the plane of the first segmentation passes through it.*

The author agrees with Fol that the vitelline membrane is not preformed, but arises with the penetration of the first spermatozoön, and thus offers an insurmountable obstacle to the penetration of other spermatozoa.

Kupffer und Benecke (**'78**, p. 21) maintain that in the case of Petromyzon Planeri and P. fluviatilis there are two polar bodies (Richtungskörper) eliminated, one before and one *after* fertilization. As regards the former of these, it was first observed after the retraction of the vitellus,† and therefore its origin and the method of its formation were not observed. It was entirely overlooked by both A. Müller and Calberla. Kupffer and Benecke say (p. 16) that it gives the impression of a nucleus which is surrounded by a small portion of a coarsely granular mass. Often a distinct nuclear membrane is to be seen, and sometimes within it a highly refringent nucleolus; more often, however, only fragments of a nucleolus. They think it comes from the substance of the disappearing germinative vesicle, either before or during fecundation. It is applied to the inner surface of the watch-glass-shaped elevation of the egg membrane,† but never at the highest point of the dome; and when the micropyle is eccentric, it is found on the side of the dome opposite the latter. The authors combat the view entertained by Calberla, that the germinative vesicle gives place to a female pronucleus at the time of the metamorphosis of the "Ammocetes" into the adult. "Aber diese Auffassung (Calberla's) verliert allen Boden durch den von uns geführten Nachweis, dass *am Beginne des Befruchtungsactes* ein Richtungskörper eliminirt wird" (p. 20). The proof is not entirely satisfactory to me, for I do not see what direct evidence has been produced to show that the supposed polar corpuscle may not have been eliminated from the yolk at a much *earlier* period than that of fecundation. That it might after elimination become enveloped by the yolk, — which before fecundation fills completely the egg membrane, — and thereby

* The signification of this "Dotterhügel" and its relation to the first plane of segmentation will be discussed hereafter. See p. 499.

† See the account given elsewhere (p. 501) of the changes accompanying fertilization.

escape observation, cannot be considered strange, since similar changes resulting in the obscuration of polar globules have been frequently observed. *Subsequent* statements furnish the only ground presented for such a conclusion. The authors found, namely, on eggs taken from females ready for oviposition, that there was constantly a large, flattened lenticular nucleus near the active pole in the superficial layer of translucent protoplasm. This is comparable, they believe, with the germinative vesicle of birds' eggs, and with that which O. Hertwig has figured for mature batrachian eggs ; it is, however, smaller than the latter, but larger than, and *not* comparable with, the deeply situated nuclear structure (Eikern) shown by Calberla in his Figs. 3 and 4. This germinative vesicle, from its position and size, just covers the dark spot called by Calberla "inner micropyle." After fertilization the place of the vesicle is occupied by a clearer mass, but it is difficult to determine its limits on hardened eggs.

Before the protoplasmic "Zapfen" ("Dottertropfen" of Calberla) disappears, one observes that a *globular, granular body* arises within its previously clear mass, and that it is ejected (second polar globule) from the "Zapfen" as the latter sinks again into the yolk.

In Clepsine the germinative vesicle gives place, according to Whitman (**'78**[a], pp. 13–49, Figs. 1–9, 60–67), to a bistellate figure, which is called "archiamphiaster," while the egg is still in the ovary. The details of the process were not observed. In the earliest stages seen the axis of this archiamphiaster is inclined to that radius of the egg which passes through the centre of the amphiaster, but later this obliquity disappears, and the axis of the figure coincides with the radius. The most conspicuous parts are the two poles, encircled as they are with well-defined radial lines which extend out into the densely packed yolk spheres some distance beyond the polar "areas." The central part of the area is more deeply colored with carmine than its peripheral part. Between the two poles is a more or less spindle-shaped space free from yolk spheres. This corresponds very nearly with the germinative vesicle in size. Within this space the radial lines of the two stars are continuous from pole to pole. These interstellate lines appear to differ in no essential way from the other radial lines. In only two preparations was anything found comparable to Strasburger's Kernplatte, and in these cases of so doubtful a character that they were omitted from the drawings. Whitman is inclined from this to regard with favor Fol's idea that the spindle fibres are identical with the stellate rays, and only appear different since they are surrounded by different media. The

archiamphiaster is already formed at the time of extrusion, and usually has a radial position with one pole so near the surface that it gives rise to a "polar figure" visible on the living egg as a white spot with distinct radial structure. After the archiamphiaster is formed, the egg, provided it is not extruded and brought in contact with water, may remain in a quiescent condition for at least two (or perhaps for even four or five) days, without any injury or abnormal effect upon its development. There appears in the centre of the "polar figure" about half an hour after extrusion a minute *pellucid spot* which is entirely free from yolk spheres and granules. This is the *central part* of the polar area of the outer star, and is deeply stained in carmine. I have elsewhere (p. 421) alluded to the significance of this pellucid spot. Although the subject is not formally discussed by the author, it seems to me that he leaves the impression that he regards this polar corpuscle "*C. P.*" as the beginning of the new nucleus. At least, he says, a similar "pellucid spot" is seen immediately after the formation of the second polar globule, and marks the place of its exit (p. 20). A section of the egg at this time shows beneath the globules a circular space free from deutoplasm, open toward the globules, and filled with a very fine granular substance, which has the lead-gray tinge characteristic of the germinative vesicle that has been treated with osmic acid. This body, which appears as a *pellucid spot* on fresh eggs and which may be designated with Van Beneden and Fol as *female pronucleus*, says Whitman, is the remnant of the archiamphiaster. Thus indirectly we may infer, I think, that the first-mentioned "pellucid spot" was estimated by him to be a nuclear structure. As far as I can judge by comparison with other objects, I am inclined to think that no part of the *Kernplatte* is embraced in these pellucid spots. I am not so confident that no part of this areal corpuscle enters into the composition of the female pronucleus in the case of Clepsine. To judge from what takes place in Limax, it is to be expected that this corpuscle in the polar cells, at least, takes no part whatever in the nuclear structure. If it shall hereafter be possible at any time to trace the fate of the Kernplatte, the question may be definitely settled; till then I can only believe that there is no essential variation in Clepsine from what I have seen in Limax.

The formation of the polar globules in C. marginata is accompanied by a very interesting change in the form of the egg, first observed by Whitman. About thirty minutes after extrusion a marked constriction of the egg at the equator becomes visible; this constriction without becoming very deep advances slowly and uniformly toward the pole where

the pellucid spot is located. In from ten to fifteen minutes it is completed, leaving only a nipple-like protuberance from which the first polar globule begins to emerge. "That part of the polar globule first to appear is perfectly transparent, but the half last eliminated is filled with minute, highly refractive granules, the outer border of which forms a straight line at first." After its elimination, the yolk, which had receded from the vitelline membrane at the formative pole of the egg; again fills out the perivitelline space coming in contact with the membrane, and thus the polar globule is pushed so far back into the yolk that it is seen with difficulty. A similar, but not so marked or regular, peristaltic constriction accompanies the formation of the second polar globule. In C. complanata the furrow often appears raised in the middle, giving it the appearance of being double. It is possible that the same phenomenon has been fixed by reagents in the Limax egg shown in Fig. 55.

The fate of the germinative vesicle and the significance of the polar globules are discussed by Whitman at some length. The germinative vesicle is not totally eliminated, so there is really no enuclear or cytode stage, which, moreover, from *a priori* grounds could hardly be expected. "Ontogeny furnishes numerous examples of reversion, but I believe no case in which reversion is followed by progression to the same point again." Although the genetic connection of the archiamphiaster and the germinative vesicle were not absolutely demonstrated in Clepsine, yet, granting this, "the proof in Clepsine is as complete as it well can be for opaque eggs that a part of the germinative vesicle persists as a nuclear element" (p. 34).

The occurrence of polar globules the author thinks still a matter of doubt in birds, reptiles, amphibians, most fishes, tunicates, arthropods, and rotifers. I have shown it to be highly probable, however, that Strasburger has seen stages initiatory to the formation of a polar globule in Phallusia.

Whitman maintains that it is impossible to make a direct comparison of the elimination of the entire germinative vesicle, as represented by Balfour and Oellacher, with the formation of polar globules by amphiastral division. The "pole-cells" in insects, as they form the basis of the sexual organs, cannot be equivalent to polar globules; nor can the so-called "testa-cells" of the ascidian egg.

Perhaps the most interesting part of Whitman's discussion is that which considers the *historic origin* of the polar globules (pp. 44 – 49), to which the reader must be referred, since there is space here for only a

brief account. The objection to Bütschli's theory, that the formation of polar globules is equivalent to the elimination of the "nucleolus," which occurs in many Infusoria as a result of (temporary?) conjugation, is found in the fact that the polar globules are formed *independently* of fecundation, while the "nucleolus" of Infusoria is ejected as a *consequence* of the conjugation.

The view held by Bütschli, that the production of polar globules is a process by which the nucleus is rejuvenated, — a phenomenon, not of the maturation of the egg, but of the earliest phase of its development, which may take place either parthenogenetically, or under the influence of fecundation, — and therefore that the meaning of this process is to be sought *in the elimination of a part of the egg nucleus*, is not, according to Whitman, the interpretation "most in harmony with the phenomena of conjugation, the characteristic feature of which is the *addition* rather than the *removal* of substance." For this reason the forms both of total and of temporary conjugation observed among Infusoria are fundamentally the same, the latter being, so to speak, an abridgment of the former.

"Impregnation in both plants and animals consists," says Whitman, "in a complete and permanent fusion between corresponding parts of two unicellular individuals, fully analogous to what happens in the first mode of conjugation, with this difference, that polar globules and 'canal cells' are produced before the fusion begins, or at least before it is completed," but not so in the case of conjugation. "In what relation, then, do polar globules stand to impregnation?" "That there is no necessary [causal] connection is in harmony with the absence of such corpuscles in conjugation." A temporal relation, however, does exist. Whitman adopts the view which homologizes the "canal cells" of plants with the polar globules. In the former the "canal cells" stand at the end of a series of asexual generations, the impregnated egg beginning a new series that will end like the preceding. "Just as fecundation in plants is followed by cell proliferation culminating in sexually differentiated cells, destined to copulate and renew the cycle of changes, — all other products of the proliferation (canal cells with the rest) eventually dying out, — so in Infusoria conjugation is succeeded by reproduction by fission, the ultimate products of which are sexually differentiated individuals. The chief difference here is, that in one case (Infusoria) all (?), in the other only a comparatively few, individuals become capable of gamic reproduction; but this difference, having reference only to a specialization of function which necessarily accompanies the development of a multicellular organism, authorizes no fundamental distinction. In Metazoa, like-

wise, a gamic cell-generation is followed by a line of agamic generations, the last of which are the small cells called by Robin polar globules. With the production of these globules we arrive at the sexually ripe egg. In accordance with all this, I interpret the formation of polar globules as *a relic of the primitive mode of asexual reproduction*, which normally precedes fecundation, and is therefore no part of the process of impregnation. This interpretation accounts for the otherwise inexplicable fact that amphiastral divisions of the nucleus introduce the formation of the directive cells, and is in harmony with the absence of such cells in Infusoria, and their general occurrence among plants and animals."

The subject of "polar rings" is considered in connection with that of pronuclei, and both are reviewed farther on. (See p. 503.)

The second of the papers by O. HERTWIG (**'78**[a]) of which a synopsis was published in 1877 contains the results of studies on cœlentrates, worms, echinoderms, and mollusks. Among the cœlentrates the uninucleolar is the prevailing but not the exclusive condition of the germinative vesicle. As in Asteracanthion the nucleolus is composed of two substances of different refractive power. The eggs of Æginopsis and Mitrocoma when excluded are naked and agree with Toxopneustes in the early formation and loss of the polar globules, which can be found only by the study of eggs taken from the ovary. In Pelagia and Nausithoë there are two or three polar globules, which are retained in contact with the yolk by the gelatinous mass in which the eggs are laid, and which contain one or several nucleolar structures. If *three* globules are formed, the third arises by a division of the one first formed. All the eggs which are ripe and excluded into the sea-water already possess before fertilization a small homogeneous egg nucleus at the surface of the yolk.

Of the Siphonophoræ the eggs of both Physophora hydrostatica and Hippopodius gleba exhibited each two polar globules, mistaken by P. E. Müller in the case of the latter genus for spermatozoa.

Among the Ctenophoræ, Gegenbauria cordata exhibited constantly two polar globules, at some little distance from an egg nucleus which lay at the boundary of the yolk granules and cortical layer of protoplasm. A third body like the polar globules was occasionally seen a little distance from the latter, but why he should suggest that it might be a spermatozoön rather than a third polar globule, I do not understand.

The germinative vesicle of the immature eggs of Sagitta is peculiar in having, instead of a single large nucleolus, a number of smaller nucleoli which lie on the membrane of the vesicle. Also a reticular substance is

visible in the interior of the vesicle. The latter at maturity approaches the surface of the yolk and is dissolved before the egg leaves the ovarium. In eggs treated with acetic acid the Richtungsspindel was observed to have a peculiar structure. It was composed of a bundle of stout, short, lustrous rods of uniform thickness throughout, and so arranged as to appear in optical cross-section as a circle of conspicuous granules. The formation of two polar globules, and the subsequent appearance of an "egg nucleus" (at first as a small vacuole in the periphery of the yolk under the polar globules), were observed in the living egg to follow each other after intervals of a quarter of an hour only. In already excluded eggs of Alciope a maturation spindle of considerable size was observed.

The germinative dot in eggs of Ascidia intestinalis, as well as in Physophora, in Sphærechinus, and in several mollusks (Unio, Tellina, Helix), was found to be really composed of two substances, having, as in the case of Asteracanthion, different physical and micro-chemical properties. To designate these Hertwig uses the name *Nuclein*, for the larger, less refringent, and usually enveloping substance; and *Paranuclein*, for the smaller body. As the names imply, he considers the former as the essential part and the latter as the accessory part. This he does notwithstanding the fact, already established by his studies on Asteracanthion, that the "Paranuclein" (as I conclude from the account of its deportment in the two cases) is the part which is "*in erster Linie*" engaged in the transmigratory changes accompanying the formation of the first maturation spindle. Flemming, moreover, holds, as Hertwig states, the reverse opinion as far as regards the case of *lamellibranchs.*

Hertwig gives figures from his earlier studies on Hæmopis which now have greater interest in view of his observations on the starfish. They represent stages in the formation of the "Richtungsspindel" when portions of the nuclear substance are still to be found in the vicinity of the spindle figure. These bodies entirely disappear with the completion of the spindle, i. e. by the time the polar globules begin to be formed.

The formation of two polar globules in the sea-urchin (Sphærechinus brevispinosus) takes place in nearly the same manner as in the starfish, except that the two maturation spindles and archiamphiasters are larger. Hertwig acknowledges that his previous representations of the metamorphosis of the germinative vesicle were produced from eggs in a pathological condition. The reason why the maturation spindle was not previously found in mature eggs is explained by the polar globules being formed in the ovary, and at a time when the eggs do not possess a firm membrane, so that the latter are lost in the ovarial fluid.

The eggs of the sea-urchin are peculiar from the great length of time (sixteen to eighteen hours) during which they remain capable of normal fertilization. The abnormal penetration of several spermatozoa Hertwig thinks is due to the *protoplasm, impaired in its vital energies, no longer offering resistance* to such penetration.

Among mollusks the eggs of Mytilus afforded excellent results, which in the main so far corroborate the evidence of his other observations that I confine myself to a few minor points. Before the first maturation spindle has reached the surface of the yolk a corpuscle (sometimes divided into halves) is seen at some distance from the spindle. He is not quite certain, but inclines to the opinion that it consists of nuclear substance, for it disappears some time after fertilization, i. e. *before* the formation of the polar globules. It is interesting to observe that the egg does not advance beyond the formation of the first maturation spindle unless it is fertilized. Then, after fifteen minutes, the polar globules are quickly formed (the second follows the first after twenty-five minutes), and carry before them the double-contoured egg membrane. The spindle becomes *shortened* and *thicker* before the globule is formed. A prominence arises at the vegetative pole of the egg when the first cleavage amphiaster makes its appearance; it ultimately forms a part of the greater (vegetative) segmentation sphere.

The criticism I have made on the account given by Fol of the early changes in pteropod eggs, simply from a comparison with the changes which occur in Limax, is strengthened by the conclusions to which Hertwig arrives from a study of mollusks more nearly related to those investigated by Fol. It follows from Hertwig's observations on Tiedemannia Neapolitana and Cymbulia Peronii that the formation of polar globules and of the egg nucleus takes place in essentially the same manner as in Asteracanthion. The two polar globules are formed one after the other, — not by the division of a single globule. The "Verdichtungszone" of the maturation spindle may in Tiedemannia be seen in the *living* egg as a row of short dark rods.

He passes over the formation of the second maturation spindle by simply saying that the spindle-half which remains after the second polar globule is formed, completes itself again. The female pronucleus arises as a *cluster* of vacuoles. It is noticeable that in all the mollusks described by Hertwig, except lamellibranchs, it remains very near the animal pole of the egg just as in Limax, and that in all cases the female pronucleus, unlike Limax, seems to exercise less influence on the surrounding protoplasm than does the *male* pronucleus.

The phenomena in Pterotrachea and Phyllirhoë are so nearly the same that they are described jointly, and afford excellent results on the nature of the metamorphosis. The spindle is formed *within*, and therefore out of the substance of, the germinative vesicle. On preparations made with acetic acid the spindle is found to lie through the middle of the vesicle (or a little eccentric), its ends with their extensive asters lying at two poles of the vesicle where its wall has been dissolved. The coagulated nuclear fluid (Kernsaft) is distinguishable after the membrane of the vesicle has been *entirely dissolved*. When the spindle has taken a radial position the yolk exhibits a *depression* at the point where one of its ends reaches the surface. The second spindle is much smaller than the first.

While I can fully acquiesce in a majority of the points defended by Blanchard (**'78**, pp. 747–754), I cannot think all the conclusions he has reached are justified by the literature which he has so recently reviewed. It is at least confusing for him to say, "The germinative vesicle disappears, not because it is dissolved in, but because it is expelled from the vitellus, just as Pouchet maintained thirty years ago," even though he subsequently gives a less prejudiced account of these changes. It is likewise very unsatisfactory, because incomplete, to say that the germinative vesicle in escaping from the vitellus leaves behind in the yolk a part of its *fluid* (suc) in a state of solution. That I may not misrepresent the conclusions of Blanchard, I must add that he recognizes the derivation of the female pronucleus from the half of the second spindle which remains in the vitellus *and "se désorganise."* I do not understand how a process of *disorganization* can result directly in the construction of a new nucleus, and cannot share the belief that the spindle metamorphosis of the germinative vesicle is "a consequence of its natural death," since thereby I should be compelled to look upon the spindle metamorphosis which accompanies every subsequent cell division — although presenting the most striking evidence of activity — as a consequence of the death of the nucleus! One should not maintain, as Blanchard does, that the polar globules exercise a considerable influence on the direction of the segmentation furrows and the reciprocal relations of the blastomeres. It cannot be doubted that there exists a constant spatial relation between the polar globules and the furrows, but to seek the *cause* of this coincidence in a supposed influence of the polar globules over the position of the furrows is to adopt an explanation of which there has as yet been adduced no proof, and which is much less satisfactory than that which makes the position of the place both where the polar globules shall emerge and where segmentation shall

subsequently begin depend upon the same cause (not yet fully understood), — a cause which effects the segregation of the more active constituents of the egg about the pole in question before *either* of these phenomena have taken formal expression.

Blanchard pertinently objects to Rabl's theory of the protective office of the polar globules, on the ground that, if injurious pressure were exerted by the egg membrane, the globules would only serve to increase its damaging effect by concentrating the pressure upon a more limited extent of the embryo's surface, and thereby necessarily increasing proportionally the intensity of the pressure.

3. *Fecundation.*

It is my purpose to review such papers as treat the subject of fecundation in the light of the recent discoveries of nuclear copulation, or such as have paved the way to so fundamentally important a conception of the nature of the process in question. The order in which these phenomena have been discovered has been nearly the reverse of the succession in which the events of fecundation make their appearance. It was in the earlier part of the present decade that a beginning was made in divesting the *later* stages of fecundation of some of their mysteries, and only by a sort of retrogressive exploration that we have within the past two or three years come to understand better the *earlier* stages of the process, and to put all in more satisfactory correlation.

Bütschli (**'73**[a]) was one of the earliest observers to trace some of the changes which overtake the pronuclei, but he could give no account of their origin, and therefore had no idea that they were intimately connected with the fecundation of the egg, as he also was in doubt about their actual coalescence. His account of the phenomena accompanying their union has been, for the sake of convenience, given in another connection (pp. 280, 396).

The studies of Weil (**'73**) I am only acquainted with through Hofmann and Schwalbe's "Jahresbericht," etc., from which it is to be learned that he has observed in rabbit eggs taken from the oviduct between seventeen and forty-six hours after fecundation (should probably read "after copulation") living spermatozoa, in four cases within the egg protoplasm itself. Like Van Beneden, Weil also saw two nuclei (male and female pronuclei) before the beginning of segmentation.

What has already been said of the origin of the female pronucleus, as described by Auerbach, is true of the male pronucleus. Concerning the further changes of the pronuclei after they meet in the centre of the

egg, AUERBACH (**'74**, pp. 210–217) says that they continue their motion until they become, to a considerable extent, mutually flattened. The line of contact is very fine, and the failure of the nuclei to melt together at once is due to that condition of the surface of the two nuclear drops known to physicists as superficial tension, and not to the existence of a veritable nuclear membrane. The flattened pair of nuclei soon commence a rotary motion around an axis perpendicular to the long axis of the egg, which continues till the plane of separation, which originally was perpendicular to the long axis, comes to lie parallel with it. The rotation, like the migration of the nuclei, is passive, i. e. is effected by the contractility of the protoplasm. Toward the end of this rotary motion the nuclei become more flattened, and the nucleoli become, one after the other, gradually paler and somewhat larger, and then suddenly their substance scatters, forming a cloud, which almost immediately vanishes. After this the line of separation suddenly disappears along its whole extent, and the two nuclei are one. If it were a layer of protoplasm or a membrane which separated the nuclei, it could not disappear throughout its entire length at the same instant. The single nucleus by elongation now assumes a rhombic or broad-spindle form.

When he comes to an interpretation of the meaning of this melting together of two nuclear structures, Auerbach believes that it is to be understood as a sort of conjugation (pp. 248, 249), — a necessary introduction to the process of successive nuclear increase, which is soon to follow. Hence it is a kind of nuclear reproduction. Just as for the reproduction of individual organisms a copulation of two individuals is so often indispensable, so for unicellular organisms is that of two *cells*. Every conjugation has manifestly for its end the improvement (by a process of intermingling) of individual peculiarities, — the mutual complementing of deficiencies. A difference in the two uniting elements, so common elsewhere, is not wanting here. The difference in the place of origin of the polar nuclei — the one at the smaller pole where the spermatozoa penetrated, the other at the opposite pole — will influence the *quality* of the nuclear material and induce one-sided faults in the composition of each. To correct this is the object of the migration and confluence of the primitive nuclei. But if these were simply to meet and coalesce, then, owing to the inability of the thick nuclear fluids immediately to intermingle, the whole process would be futile, since with the first segmentation each half of the nuclear mass would be relegated to the half of the yolk in which it arose. This is obviated by the rotation of

the mass through 90°, whereby each half supplements the half of its own nuclear fluid by the half of that which arose at the opposite pole of the egg.

In Cephalobus rigidus Bütschli ('75, p. 202) says he has seen the process of fecundation in the most satisfactory manner. As soon as the egg reaches the first spermatozoön of the seminal vesicle it unites with it at once. The spermatozoön attaches itself closely to the surface of the yolk, and when the latter has entered the uterus appears already fused with it. The egg certainly combines with no other spermatozoön in its passage through the seminal vesicle. In Cucullanus the egg at the moment of fecundation was not observed, but fecundated eggs disclosed clearly the entered spermatozoön as a *cluster of dark granules surrounded by a clear area.* It is therefore not at once fused with the yolk in this case. The results reached in this preliminary account regarding the origin and fate of the pronuclei I have given in connection with the subject of maturation (p. 403). Bütschli fails to connect either of the pronuclei *directly* with the penetration of a spermatozoön, but attributes the beginning of the maturation phenomena to the influence of fecundation. Since by the ejection of the polar globule a component of the nucleus is removed, it is readily to be inferred that the same is replaced by components of the spermatozoön, especially since subsequently (during segmentation) a part (spindle) corresponding to the polar globules is found in the nucleus. There is ground for the statement that the essential thing in fecundation consists in the removal of the old *nucleolus*, and the formation of a new one to which elements of the spermatozoön contribute (p. 210).

For Bombinator Goette ('75, pp. 51 *et seq.*) describes the disappearance of the germinative vesicle, which leaves behind for some time a starlike figure in the upper half of the yolk. Immediately after fecundation in the more advanced eggs, a "yolk nucleus" (Dotterkern) has already made its appearance near the middle of the egg as a large, round, somewhat flattened body, with distinct but not sharp contour. The finely granular substance of the disintegrated germinative vesicle reaches within its territory, but with such want of uniformity as to justify the assumption that the two structures sustain only a chance relationship. This "Dotterkern" migrates toward the upper pole of the egg, while the discoloration of the yolk, due to the disintegration of the germinative vesicle, disappears entirely; and thereupon is formed within it a delicate round corpuscle — the first "Lebenskeim" — which induces the further development of the egg. This "life germ" per-

sists when, soon after, the yolk nucleus becomes faintly outlined and disappears.

As I have elsewhere indicated, it is probable that FOL (**'75**[a], Pl. VII. Fig. 2, and Pl. VIII. Fig. 2) saw and figured for Pteropoda, without comprehending its true significance, the *male* pronucleus, both some time before and also when it was about to join the female pronucleus, in the former case as the centre of a well-expressed aster.

O. HERTWIG (**'75**, pp. 378–398, Taf. XI.) was the first to definitely connect one of the pronuclei (Spermakern) with a spermatozoön. In from five to ten minutes after artificial fertilization of the eggs of the sea-urchin there appears near the surface a small clear space from which the yolk granules have disappeared. This space increases a little in size, and at the same time the neighboring yolk granules assume a radial arrangement about it as a centre; at first limited to its immediate vicinity, but gradually becoming more extensive and more distinct. A small homogeneous body makes its appearance in this space, from which it only slightly differs in its refractive power. Sometimes a delicate line was seen stretching from this body to the periphery of the yolk, whence it continued into the perivitelline space as a fine thread. This radial figure migrates rapidly (requiring only about five minutes) from the periphery to near the centre of the egg; here the corpuscle encounters the "egg nucleus" (female pronucleus), which has meantime slowly approached the stellate figure. The egg nucleus has a diameter of $13\,\mu$; the corpuscle, of $4\,\mu$. The nucleus now undergoes a slight amœboid change of form, both structures become less distinct, and the smaller finally disappears. A little later the limitation of the egg nucleus again becomes distinct, but the smaller body is not to be seen. The nucleus is larger than before, and of spherical form. Meanwhile the stellate figure, in which the egg nucleus has now come to lie, has increased in extent till its rays reach nearly to the periphery of the yolk on all sides.

The use of osmic acid and Beale's carmine confirms the results of these observations on living eggs. The stellate figure is, however, *less* conspicuous than in the fresh condition. By this treatment it is found that both the egg nucleus and the central corpuscle of the stellate figure become deeply stained. This warrants the conclusion that both consist of nuclear substance. The corpuscle is a little more intensely colored than the egg nucleus, a condition to be accounted for by the more compact condition of its substance. Furthermore, stages in which the two nuclear structures are in contact, and later such as show only a single nuclear

structure, justify the opinion that *the single nucleus found in the egg immediately before segmentation, and surrounded by rays of yolk granules, is the result of the copulation of two nuclei.* Hertwig also reports that, while in most cases only *one* clear spot makes its appearance in the periphery of the yolk, occasionally more (up to four) have been observed to make their way to the egg nucleus; but after the appearance of anomalous nuclear figures, the eggs soon perished. It is therefore probable that these eggs were from the beginning pathologically altered.

In the interpretation of these observations he concludes that the constancy of their appearance at a uniform interval after the mingling of the sexual elements is evidence that they are dependent on fertilization. From this and the observed filament it is not to be doubted that these changes are referable to the penetration into the yolk of a spermatozoön, of which the tail is the observed filament, while the head (its nucleus) becomes the "Spermakern." The tail is probably dissolved either at once or during the migration of the sperm nucleus. The homogeneous protoplasmic area and the radial figure are apparently induced by the sperm nucleus which occupies their centre, in the following way: the nucleus exerts an attractive influence on the homogeneous components of the yolk, which thus become most densely collected around the nucleus, and thence radiate in all directions. The yolk granules passively assume a position in the interstices between the rays of the attracted substance.

The most important part of fecundation, hitherto explained as the copulation of two cells,* is found in the fusion of the two nuclei from which "arises first a nucleus (nucleus of the first cleavage-sphere) equipped with living forces, which effectively stimulates, and in many respects controls, the further process of development in the yolk."

In a foot-note (p. 386) Hertwig calls attention to the fact that for the time being the egg cell may be considered as in an *hermaphroditic condition*, inasmuch as two sexually different nuclei are present in a common protoplasmic mass. Further, since the "nucleus" and the "nucleolus" of Infusoria are, from the changes they undergo in reproduction, comparable with the egg nucleus and sperm nucleus respectively, it follows that the Infusoria may be considered as *hermaphroditic unicellular organisms*, inasmuch as the sexual differentiation of the nuclear substance, which has been accomplished in other organisms in *two separate* cells, is with them effected in a *single* cell.

In a foot-note Bütschli (**'75**[b], p. 109) says his recent studies tend to

* See Haeckel **'74**, pp. 135 – 138, and **'75**, pp. 482, 483.

confirm his opinion that the essence of fecundation consists in a total or *partial* renewal of the nucleus of the egg cell.

Hensen ('**75**, p. 238, Taf. VIII. Figs. 5–8) never saw "ein Samenfädchen in den Dotter hinein kriechen," but has often seen these corpuscles imbedded, either entirely or the head only, in the yolk in the case of the guinea-pig and the rabbit, and draws the general conclusion (p. 241) that in the case of these animals more than one spermatozoön can penetrate the yolk, where, under definite formal changes of the head, it is dissolved, and that in this manner the fecundation of the egg is accomplished.

Ed. van Beneden ('**75**, pp. 693–695) was never able to observe the penetration of a spermatozoön into the vitellus of the rabbit's egg; but from often finding spermatozoa very closely adherent to the surface of the yolk, he ventures to express the belief that "fecundation consists essentially in the fusion of the spermatic substance with the superficial layer of the vitelline globe." His account of the formation and union of the pronuclei is given on pages 412 to 414.

The penetration of spermatozoa into the egg, which Robin maintains ('**75**, p. 21), does not imply a penetration into the yolk substance. The ultimate molecular union of the substance of a large number of those which penetrate the membrane and are liquefied, is evidently only an inference from a supposed diminution of those still found in the perivitelline fluid at later stages (see Robin '**62**, p. 87). "The retraction of the yolk, the changes which supervene in its granules, the formation of polar globules, are partial phenomena which occur with eggs whether fecundated or not; but the production of the *vitelline nucleus* only takes place in ovules into which spermatozoa have penetrated, i. e. [in ovules] to the vitellus of which male substance has been united." ('**75**, p. 86.) Notwithstanding the accuracy of the greater part of this statement, it does not follow that the author understood the true origin of the nucleus of the first segmentation sphere,—his "noyau vitellin." In fact, it has in his opinion an origin entirely independent of the germinative vesicle, at the centre of the yolk, by a molecular association of "principes immédiats" of the vitellus. It is with the appearance of this nucleus that the ovule takes on the characters of a new being, and ceases to be an anatomical element of the adult animal which produces it.

Thus, of all the parts which compose the ovule before maturity, the vitellus, he believes, is the only one which serves for the production of a new being.

I shall not reproduce the second part of Van Beneden's ('**76**[a], pp. 76–

83, and **'76**^{b}, pp. 178–182) paper * on the germinal vesicle and the first embryonic nucleus, for it is an attempt to harmonize Hertwig's observations on Toxopneustes with the author's own studies on mammals, which was only made possible, as Hertwig (**'77**, p. 77) himself has very clearly shown, by a misconception of the account given by the latter. Van Beneden's assumption that Hertwig's "Spermakern" is a *nucleolus* finds no support in Hertwig's description, and the *protoplasmic area* surrounding it is certainly not a nucleus, and therefore not comparable with Van Beneden's "pronucleus périphérique." While there is no reason to question the interpretation which Van Beneden assigns to his own observations, his attempts to subject Hertwig's observations to an unnatural alliance with his own must be regarded as unsuccessful.

In the egg of the common toad after fertilization Van Bambeke (**'76**, pp. 117–135, Pl. II.) has observed that meridional sections exhibit, instead of a single pigmented trail, — the claviform body of the unfertilized egg, — *two* such trails. One of these, the "traînée en boudin," is slightly swollen at its internal end, and reaches nearer to the centre of the yolk than the second, — "traînée triangulaire," — about the inner end of which it is curved as about a centre. At its periphery it abuts upon the germinative fossa. This the author thinks is unquestionably the claviform figure of the unfertilized egg made to take a curved course by the pushing in against it of the second or triangular trail. The latter is also mingled at its base with the pigmented cortical layer of the superior half of the egg; its apex is directed inward, and is slightly curved upward so as to terminate in the space surrounded by the curved "traînée en boudin." In the terminal part of the triangular trail was once seen a clear homogeneous point limited by a strongly pigmented contour, which the author considers the nucleus of the first segmentation sphere.

Similar conditions are found in fertilized eggs of Pelobates. Here, however, the claviform figure is not curved, and its inferior enlargement, in place of being a pigmented mass, is less deeply colored than the zone which immediately surrounds it. The apex of the triangular trail, having come to occupy the centre of this enlargement, is seen to abut upon an elliptical nuclear mass (nucleus of first segmentation sphere), which is a little clearer than the surrounding yolk, and is limited by a pigmented contour, whence granular striations of the yolk radiate.

Observations of a similar kind on the eggs of the Axolotl convince the

* The English translation of this paper (**'76**^{b}) exhibits an omission which is of rather vital importance to Van Beneden's argument. It may be corrected by inserting, in the 15th line from the top of p. 181, "*nuclei* of the" before "cleavage spheres."

author that the triangular trail of the tailless Batrachia is homologous with that which takes its origin from the "trous vitellins," as previously described by him ('**70**, pp. 64, 65). Finally, after a review of the literature, Van Bambeke arrives at the conclusion that the eggs of the Batrachia, immediately after impregnation, still embrace traces of the claviform figure, but nothing discloses the presence of the "Eikern" of Hertwig, or the "pronucleus central" of Van Beneden. The nucleus of the first segmentation sphere arises from the periphery; it very probably results from the penetration into the vitellus of a spermatozoön, which leaves as a trace of its passage the "trou vitellins" and the "traînée pigmentaire."

In Nephelis, after the conversion of the germinative vesicle into a nuclear spindle, Bütschli ('**76**, pp. 216, 217) has seen a little elevation of clear protoplasm near the animal pole of the yolk, and believes it is caused by the union of a spermatozoön with the yolk, during which the spermatozoön, possibly by swelling, has become metamorphosed into the protuberance.

Other phenomena, which Bütschli did not think of connecting with fecundation, are probably phases of that process. The "third" system of rays about a homogeneous area we may now safely infer to be the male aster. The relation which the nucleus (male pronucleus) sustains to this "area" deserves attention. According to the text, "it always lies nearly in the *periphery* of the central area." From Fig. 3, Taf. I., it is evident that it lies in that part of the periphery *nearest* the female pronucleus. I do not know of any other observation which agrees with this in the particular last mentioned. In eggs of Cucullanus that have passed the seminal receptacle (p. 223), a clear corpuscle, which encloses a cluster of granules, is found imbedded in the surface of the yolk. It is, says the author, the result of the union of a spermatozoön with the yolk (see Taf. III. Figs. 1, 7, 12). It, however, disappears before the beginning of the formation of new nuclei. The latter arise close under the surface of the yolk, and are from the beginning distinctly vesicular, with dark envelope and granular contents, but never acquire a special nucleolus. They migrate toward the centre and become fused into a single nucleus. In Anguillula rigida (pp. 232, 233) the egg unites with the first spermatozoön with which it comes in contact, but never with a second.

In the mollusks studied (p. 238) the first evidence of the existence of a male aster was observed (Lymnæus) when the first polar globule had been eliminated. It already occupied the centre of the yolk. A nucleus

(male pronucleus) was not observed in connection with this aster. From among the large number of nuclear structures (nine in Lymnæus) that appear in the yolk under the place of the polar globules in Bütschli's figures, it is not possible to say always which represents the male pronucleus, though there is usually one (Taf. IV. Figs. 7 – 9) which from its deeper position or larger size may perhaps be inferred to be such.

In Succinea the pronuclei closely resemble those which I have found in Limax, the membrane (?) being much wrinkled by the action of acetic acid. In Fig. 23 of Bütschli's Taf. IV. the nuclei occupy a peculiar position, their plane of contact lying in the animal radius of the yolk. I have never seen just such a relation. Whether in Succinea the male pronucleus is at any time surrounded with a radiate structure of the yolk, does not appear from Bütschli's studies. I am inclined to think it may be wanting, as in Limax. In view of the possible absence of stellate figures in these cases, it still remains with me, as it was with Bütschli, an open question, whether the central stellate figure of his Fig. 4 is really a newly formed aster. Against the probability of its having anything to do with the male pronucleus, it may be urged, in addition to the probable absence of a male *aster*, that no nuclear (vacuolar) structure was observed in its immediate vicinity, and that the aster occupies the centre of the yolk at so *early* a stage. Bütschli evidently inclines to the opinion that it has no genetic connection with the first spindle. If he is right, then it must be regarded as the male aster; but I am inclined to believe, for the reasons just given, that it is the deeper star of the second archiamphiaster, whose spindle has not been distinguished.

Bütschli endeavors (p. 391) to connect the "Neubildung" of nuclei in the first segmentation sphere with the segregation of very clear nearly homogeneous protoplasm. It is usually collected at the place where the polar globules emerge, but it may be more widely distributed over the surface, and may even (Nephelis) collect at a point within the yolk. This clear protoplasm forms the centre of a system of rays, and *within* it the new nuclei arise from very minute beginnings. These beginnings are small compact corpuscles (p. 408) which rapidly become differentiated into small vesicles. Just as in the formation of the nuclei in cell division, so here the simplest primitive form is farthest from Auerbach's conception, — an excavation in the protoplasm filled with a fluid, — it is a homogeneous, compact condition. Since each of the several nuclei possesses the same histological structure as the nucleus which results from their fusion, there is no ground for uniting with Selenka in calling the former "nuclear *germs*," nor for saying, with Strasburger, that they are

not so many individual nuclei, but that they furnish the material for the construction of a nucleus.

A comparison with the conjugation of Infusoria leads Bütschli to the conviction that in the fecundation of the egg similar modifications — "total or partial renewal of the nucleus, or a material revival of the same by the importation of a new part" — may be encountered (p. 438). The two nuclei (pronuclei) are alike, and arise in the same manner. There is not the least justification for interpreting them as egg nucleus and sperm nucleus in O. Hertwig's sense. The existence of a multiple of nuclei is a phenomenon induced by the antecedent subdivision of the nucleus of a spermatozoön which penetrated the yolk, not by the penetration of several spermatozoa, as O. Hertwig concludes.

To ascertain whether the formation of polar globules is dependent on fecundation the author instituted experiments on two nematodes (Rhabditis teres and R. pellio) rearing *isolated* females. The eggs never produced polar globules, and no changes of any sort overtook the germinative vesicle or dot until the yolk began to show signs of degeneration. Bütschli concludes (p. 442) that both views are warranted, — that in one case it is in consequence of, and in another independent of, fecundation, but it is not a phenomenon of the maturation of the egg; it is one of the first of the phenomena of development, which in certain cases may take place *parthenogenetically* before fecundation.

Strasburger ('76, pp. 21, 295, Taf. II. Figs. 19 – 23, Taf. VII. Figs. 9 – 11) has given the following account of fecundation in Picea vulgaris after the formation of a canal cell which remains in close contact with the ovum. The pollen tube, making its way through the disorganized cells of the neck of the archegonium, destroys the canal cell and reaches the ovum, where its previously dissolved contents pass by a diosmotic process through its very porous tip into the interior of the ovum, and are taken up by the nucleus of the latter. This may take place in a continuous manner, or the contents may first be accumulated in a nucleus-like structure at [i. e. outside] the end of the pollen tube, and then advance to the "Eikern," or finally several such nuclear structures may arise at the pollen tube and be successively received by the Eikern. The latter, thus fecundated, Strasburger calls the germ nucleus, Keimkern; it soon begins to disappear by a radial distribution of its mass in the plasm of the egg.

His studies (p. 306) on the fecundation of animal eggs were made upon Phallusia mammillata. In the first edition of this book Strasburger held that the "Keimkern" (segmentation nucleus) took its

origin from the cortical layer (Hautschicht) of the yolk. This he now corrects, and says the error was due to the "Eikern" lying in this case (Phallusia) so near to the surface.* After new observations he accepts the views of Hertwig in holding that the "segmentation nucleus" arises from *the fusion of two nuclear structures.* The sperm nucleus, which is smaller than the egg nucleus, makes its appearance, according to Strasburger, in $1\frac{1}{2}$ or 2 hours after artificial fertilization, close to the outside of the "Eikern" between the latter and the Hautschicht, *op. cit.*, Taf. VIII. Fig. 4. It is at once surrounded with homogeneous protoplasm, which is continuous on its peripheral side with the "Hautschicht," and causes here a slight elevation of the surface. Rays emerge from this homogeneous protoplasm, but around the egg nucleus there are none. In another hardened egg (Taf. VIII. Fig. 5) he finds a *larger single* nuclear structure surrounded with rays, and concludes that it results from the fusion of the germ- with the egg-nucleus. He does not, however, agree with Hertwig, that the egg nucleus is the germinal dot, and from a review of the studies of others on animal eggs concludes "dass es sich auch in den Fällen der Erhaltung des Eikernes nicht um diesen Kern als morphologisches Element, sondern nur um dessen Substanz handle" (pp. 311, 312). An entirely parallel view is held touching the method of the formation of the sperm nucleus. In the case of Phallusia it is quite possible that the substance of the spermatozoa "diffundirt" through the egg membrane, and re-collects within the yolk to form a sperm-nucleus. In fecundation, then, it is probably a question of the introduction of nuclear substance into the egg, yet only as a *physiological* element, not of the introduction of the nucleus of a spermatazoön as a *morphological* element.

"With the greatest care to prevent fertilization," GREEFF (**'76**[a]) has raised the larvæ of Asteracanthion rubens. The only difference between the development of fecundated and unfecundated eggs consists in the tardiness with which segmentation takes place in the latter case, — it being ten to twelve hours after exclusion, instead of one to two hours, as in fecundated eggs.

In his account of the development of Heteropoda, FOL (**'76**, pp. 113, 144) says that what remains of the star after the emergence of the polar globule again approaches the centre of the vitellus and becomes rounded into the form of a nucleus; near the opposite or nutritive pole a second nucleus appears, which also moves toward the centre. These nuclei em-

* I have elsewhere (pp. 420, 421) shown how probable it is that Strasburger has in some cases confused another structure with the "Eikern."

brace nucleoli which become visible by the use of reagents. The nuclei fuse and thus give rise to the "nucléus secondaire, c'est-à-dire au nucléus du vitellus fécondé et débarrassé des matières de rebut." The source of the second nucleus remained unknown to Fol, as clearly follows from what is said at p. 144 : "From all these references, added to the results of my own observations, it appears to follow that the vitellus possesses after fecundation a central nucleus the origin of which is unknown."

I have not had access to the original paper by Giard (**'76**), but according to R. Hertwig's abstract Giard (**'76'**) defines fecundation to be a copulation of the amœba (or amœbæ) which is formed by the penetration of spermatozoa into the egg, with the egg amœba which at this moment relinquishes its encysted condition (disappearance of the germinative vesicle).

In a rabbit killed twelve hours after coitus, but not studied till ten hours later, — the sexual organs having been maintained at a temperature not above 19° C.,—Campana (**'77**) found some spermatozoa fixed in the superficial layer of the vitellus, and two still actively swimming about in the perivitelline fluid. (!)

Fol (**'77**) confirms O. Hertwig's observations of the penetration of a spermatozoön into the vitellus ; the body of the spermatozoön appears to fuse with the vitelline protoplasm to form a clear spot, which becomes the centre of a system of radial striæ. This Fol calls the *male pronucleus*. He also reports similar discoveries for other animals. In Sagitta and various Gasteropoda there is formed at the moment when the polar globules appear, and at the opposite pole of the yolk, a clear spot, surrounded, in the case of Sagitta, with a star of protoplasmic filaments. This spot moves toward the female pronucleus. During this motion one sees very distinctly that the *centre of the star is in advance of the clear spot*, and that the latter is drawn along in a passive manner. The female pronucleus remains stationary till the clear spot is near at hand ; it is then attracted toward it, and the latter at the same time moves more rapidly. The female pronucleus and the clear spot fuse to form the nucleus of the fecundated egg. The direct evidence is wanting, but to judge by analogy the clear spot is a male pronucleus.

This communication is particularly important, since it directs attention to the relation of a pronucleus to its aster. I can to a certain extent confirm this observation for Limax (see Fig. 68). It is of further interest because it presents so just an estimate of the composition of the polar globules. Still, the idea of their formation by a process of *cell division* can hardly be said to have been fully grasped, to say nothing of the

absence of such convincing proof of their nature as that soon brought forward by another observer.

The fecundation of Asterias glacialis, as described by FOL (**'77**[a], p. 359), is of great interest. The spermatozoa come in contact with the egg and remain with the "body" imbedded in the mucous envelope which surrounds the yolk. When one of them has succeeded in traversing half the thickness of this envelope the protoplasm of the yolk accumulates at the nearest point of its surface as a thin hyaline layer, which soon rises in the centre in the form of a boss. This next changes to the shape of a cone, and soon a fine thread of protoplasm establishes a connection between the summit of the cone and the body of the spermatozoön. The latter elongates, and, as it were, glides into the yolk, the cue alone remaining outside, where it can be distinguished for some time. Meanwhile the superficial hyaline layer increases in extent and finally envelops the whole yolk. At the moment the connection with the spermatozoön is established, this layer becomes clearly differentiated and begins to detach itself from the vitellus as a vitelline membrane. This differentiation commences at the point of fecundation, where there is formed a sort of minute crater, and thence passes entirely around the yolk. In eggs that are quite mature and fresh, these changes succeed each other with such rapidity that all spermatozoa which are a few *seconds* behind the first are debarred access to the vitellus. Fol expresses the opinion that normally fecundation is accomplished in the starfish by a *single* spermatozoön; with the sea-urchin this fact is evident. The point of penetration becomes the centre of a male aster; in the middle of which a mass is formed (the male pronucleus) which fuses with the female pronucleus as in the case of the sea-urchin. The spermatozoön exercises an attractive influence *at a distance*, as well as when in contact with the vitellus.

Toward the close of the constriction which produces the first polar globule in Nephelis, O. HERTWIG (**'77**) has observed that a small homogeneous area, surrounded by radially arranged yolk-granules, makes its appearance in the half of the egg opposite the polar globule. This subsequently takes a position in the centre of the egg, its radial system having become more extended. After the second polar globule is formed, this system becomes less distinct, and when the vacuoles make their appearance in the semi-spindle lying under the polar globules, there also appears at the centre of this central area a small vacuole. The peripheral vacuoles unite into one, and then both peripheral and central vacuoles, by the appropriation of nuclear fluid, become swollen to vesi-

cles of considerable size. By the migration of the peripheral vesicle they come in contact, and become flattened against each other. After treatment with acetic acid each appears to consist of a compact cortical layer and of fluid contents which are traversed by netlike cords with nodular swellings, and in which are found clusters of granules. This is probably an artificial production, since in preparations made with osmic acid the nuclear contents remain homogeneous, and are only limited by a somewhat firmer cortical layer. Since these nuclei were not seen to become confluent, — as is elsewhere (p. 328) more fully described, — until evidences of the first segmentation appeared, Hertwig concludes that the period of their confluence is of limited duration.

The following important conclusions are reached. The peripheral nucleus arises from the granules of the lateral zone of thickenings, as in ordinary cell-division, inasmuch as these granules are by the reception of nuclear fluid converted into vacuoles, which ultimately become fused. Therefore these vacuoles are not so many isolated nuclei (as Bütschli thinks), but the component elements of a single nuclear structure. But, as the spindle was derived from the nuclear substance of the germinative vesicle, it follows that there exists *an uninterrupted connection between the several generations of nuclei from the germinative vesicle to the nucleus of segmentation.* The direct evidence of the origin of the isolated stellate figure is wanting, but from analogy with Toxopneustes there is reason to believe that it is *produced by the nucleus of a spermatozoön which has penetrated the yolk.* Therefore *the segmentation nucleus is derived from the conjugation of two sexually different nuclei; a female nucleus, descended from the germinative vesicle, and a male nucleus, derived from the body of a spermatozoön.*

Finally, the formation of the polar globules takes place before fecundation, since the latter is really accomplished only when the confluence of the male and female nuclei takes place. This coincides, moreover, with Strasburger's studies on "canal cells." Whether the pinching off of these globules may not be affected by the act of fecundation, cannot be so positively answered.

After artificial impregnation the eggs of the frog all exhibit, according to Hertwig, a change at the pigmented pole, which is readily distinguishable with a hand-lens. The middle of the dark field appears clearer and yellowish, as though veiled in a layer of unpigmented substance. This is really a thin layer of finely granular substance (with uneven surface and thickest at its middle point) which closely resembles the contents of the germinative vesicle in its last observed stages. There are in it also

"Dotterplättchen" and fine pigment balls. He concludes that this veil is really composed of remnants of the germinative vesicle, eliminated from the yolk by the contraction of the protoplasm after the dissolution and distribution of the substance of the vesicle, and of portions of the yolk substance. But there is no ground for a comparison of this with the formation of polar globules. His own observations do not prove whether this elimination may ensue without fecundation. On eggs hardened about an hour after fecundation there may be observed, at one side of the centre of the dark field, near the margin of the veil, a "pigmented process" extending obliquely into the yolk toward the middle of the egg. The inner end of this projecting mass is swollen, and embraces a clear, finely granular substance which differs from the rest of the yolk. About this clear spot the pigment grains are radially arranged; within it is a nuclear structure of much the same nature as the pronuclei already described. This nucleus grows rapidly as the dark process lengthens toward the axis of the egg, which it finally reaches two thirds of the way from the surface to the centre of the yolk. Meanwhile a similar nuclear structure is seen near this axis in the opposite half of the egg; it is not, however, surrounded by pigment, but lies in the yolk, from which it can be distinguished only with difficulty. Subsequently both nuclei lie in the swollen end of the pigmented process, and fuse into a single nucleus which now lies immediately surrounded by a layer of finely granular protoplasm which is in turn enveloped in the swollen end of the pigment process. This requires only about two hours and a half from the time of fertilization. The interpretation which Hertwig gives these observations is too evident to require their formal statement. Never was more than one "pigment process" observed, so that the penetration of only a single spermatozoön is probably normal.

The most important fact established by Hertwig, and one entirely new for Batrachia, is the existence of an "egg nucleus" which ultimately unites with the "sperm nucleus." Concerning the origin of the former, Hertwig says, it is quite probable that so inconspicuous a structure should have existed and been overlooked before the stage at which he first saw it. From its minuteness it certainly cannot correspond to the total mass of nuclear substance contained in the germinative vesicle; that, however, does not prevent its having descended directly from such nuclear substance. The problem here is not, after all, why so little nuclear substance is transferred to the female pronucleus (Eikern), but what signification has the *multi*nucleolar, as compared with the *uni*nucleolar condition of the germinative vesicle?

In the "General Part" of this paper Hertwig considers, among other things, the grounds for maintaining the morphological identity of the "Spermakern" with the body (nucleus) of the spermatozoön, rather than the dissolution of the spermatozoön and subsequent re-collection of its substance into a male pronucleus, as held by Ed. van Beneden and Strasburger. They are: —

1. In the conifers the possibility that the fine membrane of the apex of the pollen tube is partly dissolved away, cannot be excluded.

2. In Hirudinea, mammals, etc., the vitelline membrane can present no obstacle to the penetration of spermatozoa, inasmuch as many have been observed within the membrane.

3. A difference in size between the Spermakern and the body of the spermatozoön is not evidence against their identity, since the former is by direct observation known to increase in size before copulation with the egg nucleus.

4. In Toxopneustes there is an interval of only a few minutes between the time of artificial fertilization and the appearance of the sperm nucleus. It is improbable that a solution and re-formation takes place in this short interval, nor is there any motive to such an *in*direct procedure.

5. The existence of a fine filament seen in Toxopneustes to extend from the sperm nucleus beyond the periphery of the egg, which is to be interpreted as the cilium of the spermatozoön.

PÉREZ (**'77**) gives in a note the results of his attempt to verify on Echinus esculentus Fol's recently published account of the phenomena of fecundation. In two cases he observed the protuberance of the surface of the egg, which Fol considers due to an "attraction à distance" exercised by a spermatozoön, but is unable to attribute to it the least importance. In one case there was no spermatozoön facing the elevation; in the other, a spermatozoön, after remaining immovable for some seconds in the middle of the thickness of the mucous layer, advanced actively to the summit of the elevation; but there was no delicate prolongation of the elevation toward the spermatozoön, nor did the latter glide into the yolk, — it remained fixed at the surface. Scarcely was this effected, when a second, following the same course as the first, traversed the mucous layer "with two or three leaps," and joined the surface of the minute elevation. Two others followed, but reached only the middle of the layer. Neither of the first two entered the egg, but with the elevation of the previously existing vitelline membrane, which soon followed, were borne a considerable distance from the yolk, which was, nevertheless, now fertilized.

Pérez endeavors to explain Fol's observations by supposing that the head of the spermatozoön, being a little higher or lower than the protuberance which occupied the focal plane, was "projected" upon the latter, and was thus made invisible, while the cue remained distinct. (!) This protuberance of the yolk, says the author, has nothing to do with fecundation. It is simply an accident depending solely on an interruption in the continuity of the mucous envelope, which thus forms a point of least resistance at the surface of the egg, and therefore a corresponding deformation of the yolk. Such a penetration is, moreover, an anatomical impossibility, on account of the existence of a vitelline membrane from a very early stage of egg development.

Fol (**'77**[b]) communicates interesting results concerning abnormal fecundation in the starfish, and deduces from them important conclusions. If the spermatozoa are brought into contact with eggs *before* the formation of the first polar globule, the vitelline membrane is formed and detached only very slowly around the point where the first spermatozoön penetrates, and extends over only a fraction of the surface, so that other spermatozoa continue to effect an entrance, until finally the joint result is a continuous envelope. The extent and rapidity with which this membrane is formed are proportional to the nearness with which the normal conditions are approached. The deportment of the individual spermatozoa is the same as in normal cases. The nearest male pronucleus unites with the female pronucleus, which becomes at once the centre of a system of radial filaments. The resulting "noyau combiné" unites with a second, or even a third, male pronucleus. At other times the female pronucleus, at the moment of its formation, separates into two or three fragments, which unite with as many male pronuclei. The male asters never unite with each other; they appear to repel each other, and to be attracted by the female pronucleus up to the time when the latter has been *neutralized* by union with two or three male pronuclei. When there are numerous male centres, the vitellus in its segmentation forms at once a like number of rounded elevations, — each with a male aster in its centre, — which become little spheres and continue to divide dichotomously. Thus the cleavage process is irregular, and there results an irregular blastosphere and a monstrous larva.

When the male pronuclei are limited in number and the female pronucleus is divided into two or three, the latter remain distinct. At the moment of cleavage each is converted into an amphiaster and the vitellus is divided at once into four or six spherules. Cleavage was never observed when the single nucleus resulted from the union of several

male pronuclei with the female pronucleus. A nucleus may be resolved at once into a *tetraster*, — four asters united to each other. A vitellus which has received two spermatozoa was never seen to develop normally, but always produced double the normal number of spheres.

Analogous phenomena (the penetration of numerous spermatozoa) are observable with eggs fecundated at maturity, if they have come from diseased animals. The bodies of the spermatozoa in this case remain intact within the vitellus, although surrounded with faintly expressed radial striations. As their bodies are never found intact except in these abnormal cases, Fol concludes that the male centre is produced by the *fusion* of the "body" with a little vitelline protoplasm. The mutual repulsion of the male centres he considers to be a corollary of their attraction for the female centre, just as the mutual repulsion of the poles of an amphiaster is a corollary of the attraction they exert on the surrounding protoplasm.

When the spermatozoa of Psammechinus miliaris come in contact with the egg, their heads are, according to GIARD (**'77**[a]), applied to the whole periphery of the membrane (vitelline?), and they impart to the sphere a very rapid gyratory motion. The vitelline membrane, hitherto very near the surface of the yolk, gradually separates from it, and consequently the second "cumulus" (see p. 444), whose summit adheres to the membrane, is drawn out into a cone connecting membrane and vitellus. As no spermatozoön is seen to penetrate into the vast clear space which now intervenes between membrane and yolk, the author believes that this cumulus serves for the passage of a spermatozoön; either that its summit corresponds to a pore in the membrane, or, as is more likely, that the act of fecundation consists essentially in the *diffusion* of male protoplasm through the membrane at the point where the latter is in direct contact with the female protoplasm, i. e. at the summit of the cumulus. This connecting cone soon detaches itself from the membrane, and re-enters the vitelline mass. The male pronucleus which results is not said to induce a stellate figure. The nucleolus of the male pronucleus Giard thinks cannot be the unmodified head of the spermatozoön. He is inclined to believe that the gyratory motion imparted by the spermatozoa facilitates the advance of the pronuclei toward the centre of the yolk, since eggs for some time stationary are developed irregularly. How this rotation facilitates the migration is not stated by the author.

In FOL'S (**'77**[c]) illustrated paper on the "Commencement of Henogeny," etc., it is stated that the gliding of the body of the spermatozoön

into the vitellus resembles the flow of a viscid liquid. The successive forms assumed by this lengthened "body" vary greatly in different cases, and change rapidly. It continues to diminish in size until there remains only a filament presenting varicosities and surmounted by a motionless cue. Some seconds later this latter has in turn disappeared, and one sees in its place only a very pale elongated cone. This is an exudation from the vitellus; but the vibratile cilium (cue) in process of decomposition may contribute to its formation. This "cône d'exsudation" remains visible several minutes, and assumes the most diverse forms, recalling the flames of a *feu de paille,* though not as rapid. Sometimes it is simply conical; sometimes nodulated and flanked by barbules and tongues. It finally disappears (pp. 459, 460). The rays of the male aster commence to be distinctly visible only some minutes after fecundation, and then the clear spot is already advanced a little toward the interior of the yolk. Some of the rays extend back to the surface where the contact took place, and where a minute scar still remains. Fol thinks O. Hertwig has mistaken such rays for a part of the tail of a spermatozoön. The female pronucleus commences to move toward the male pronucleus only when it comes in contact with the rays of the male aster (pp. 463, 464).

Statements relating to the fecundation of the eggs of Heteropoda are mentioned (p. 446) in connection with the review of their maturation.

Bischoff (**'77**, pp. 28–48) defends his theory of fecundation, as a *communicated molecular motion* imparted by the spermatozoön to the egg, from the misinterpretations which he holds it has suffered at the hands of those critics who could see in it *only* a "Contactwirkung." A material participation on the part of the spermatozoön does not appear to be denied, but is valueless in the author's opinion to explain the actual process of fecundation. "Was aber die räumliche oder formbildende Wirkung des Saamens bei der durch ihn auf das Ei übertragenen Bewegung betrifft, so ist diese an und für sich eine Thatsache, aber einstweilen eben nur eine Thatsache, für deren weitere Begründung und Erkenntniss bis jetzt auch nicht die mindeste Hoffnung besteht" (p. 33).

Apropos to a comparison of the ectoplasm and endoplasm of Protozoa and eggs, Minot (**'77**[b]) calls attention to the theory that "the Richtungsbläschen are comparable to the nucleoli of Infusoria. A further confirmation of this homology is offered by the formation of the 'Kernspindel' as introductory alike to the ejection of the direction cells and

the expulsion of the nucleoli." * "We distinguish therefore equally in both cases *the formation of a generation in which the two sexes are separate cells, and then the union of two sexual unicellular individuals, of different origin, to form an asexual cell, which then goes on dividing asexually for many generations* until the original energy is exhausted." The egg really becomes female only upon the discharge of the male direction cells. It is important to know whether in the development of the spermatozoa the mother cell breaks up into two portions, one of which becomes the male part, while the other remains separated. "The few available observations fulfil our expectations, for they describe a '*Mutterkern*' (female element) which remains behind and is aborted."

In a provisional theory of generation McCrady (**'77**) concludes that the act of generation consists in the actual conjugation of at least two protozoöids (one ovum, and one, or in most cases several spermatozoa). The result is twofold: (1.) the combination of the nucleus of the spermatozoön with the germinative vesicle of the ovum (this resultant is the future animal); and (2.) the aggregation of the yolk protoplasm with the protoplasm of the spermatozoa, these together constituting a store of *foo* for the immediate nourishment of the newly arisen animal, which it proceeds to appropriate in the manner of a rhizopod. This appropriation of the whole provision is the process called segmentation. It is probable that in the conjugation the germinative vesicle and the spermatozoön (or its nucleus) disappear and cease to exist as such. In their stead arises the new animal, or *protembryo.* This new animal may present itself under one or the other of three conditions: (*a*) as a clear mass of protoplasm within the yolk mass, — the embryonal vesicle of Wagner; (*b*) as a nearly uniform layer of protoplasm, completely enclosing the yolk mass; or (*c*) as a combination of *a* and *b*, in which the central and peripheral portions of protoplasm are connected with radial threads of the same. These are respectively styled *Ento-*, *Ecto-*, and *Panto-protembryo.*

With the fundamental correction now possible, that it is not the germinative vesicle, but the female pronucleus, which unites with the sperm nucleus, this view appears to approach that which Strasburger has more recently promulgated; but how far it comes short of a just appreciation of the mutual relations of nutritive substance, living protoplasm, and nuclear substance, is too apparent to demand discussion. There does not seem to have been here, any more than in the paper last reviewed,

* The nature of the argument to be drawn from the "Kernspindel" has been stated by Whitman (**'78**[a], p. 46).

an attempt made to contribute by special personal studies of the phenomena to the solution of the questions considered.

In a preliminary note on fecundation, FOL (**'77**[d]), beside communicating the substance of what has already been given, defends his conclusions from the adverse opinions of Pérez and Giard. Giard's view, that a large number of spermatozoa are necessary (by the motion they impart) to fecundation, is refuted, he claims, by his (Fol's) method of artificial fecundation, in which only two or three spermatozoa were allowed for a single egg. The cases of abnormal fecundation prove that in the eggs of this species the existence of a membrane with a micropyle cannot be admitted. If there were a membrane, as Pérez and Giard assert, there would have to be numerous micropyles.

The results communicated by O. HERTWIG (**'77**[a]) in a preliminary paper will be considered in the review of the ultimate papers (O. Hertwig **'78** and **'78**[a]) at pages 495 and 509.

The act of fecundation in Serpula — as was also stated in his previous work on the development of that animal — is, according to STOSSICH (**'77**, pp. 214, 217), external; the spermatozoön does not enter into the egg, but remains attached to its surface by means of the head, and not by the tail. The substance of the mature spermatozoön undergoes a process of transformation, by which its molecules are found in motion, which is eventually shared by the material of the egg. The movement develops itself at first in the external layer of the yolk, in the form of a rotary movement of the vitelline granulations, accompanied at the same time by a chemical transformation of the fundamental material, by which new granules are deposited and the material is rendered more opaque. In consequence of these transformations, there is secreted the gas or liquid previously mentioned by Stossich. (See p. 428.)

The discussions on the nature of fecundation in Echinodermata are further continued in "Comptes rendus," etc., by Fol on one side, and Giard and Pérez on the other. The principal points under discussion are : (1.) whether fecundation is effected by the penetration of a spermatozoön ; (2.) whether there exists a vitelline membrane before fecundation ; (3.) the nature of the vitelline protuberances called by Fol "cône d'exsudation" and "cône d'attraction."

FOL (**'77**[c]) responds by concluding that the negative results of Perez and Giard are due to their having studied only eggs already fertilized, a possibility he himself has carefully guarded against by the use of his compressorium, wherein one may observe the eggs from the first instant of the mingling of the two sexual products. Only three or four sperma-

tozoa, moreover, are allowed to a single egg. The protuberance which Pérez has seen is not at all the hyaline cone, but is a *granular* projection of the yolk of considerable size. Corresponding to the point of attachment of the ovule there is an interruption in the continuity of the mucous envelope, and into this the yolk often penetrates. This protuberance is wanting in eggs near to exclusion. In the sea-urchins the process of penetration is much more rapid than in the starfishes, and *there is no hyaline protuberance formed in this case.* Hence the error attributed to the author by Pérez is impossible, as regards the sea-urchins at least.

The pre-existence of a vitelline membrane is disproved by the author's preparations, still preserved; for when the polar globules are formed *after* fecundation, they are found to be *within* the vitelline membrane, but when before fecundation they are outside of that membrane. Fol does not deny the existence of a limiting envelope at the surface of the ovule in starfishes and sea-urchins, but it is soft and plastic, like the limiting layer of an Amœba. One can make of it a membrane by coagulating the organism. This layer normally *becomes a membrane* only at the very moment of fecundation.

Pérez (**'77**[a]) is still unable to admit an attraction exercised "à distance" by the spermatozoön, for he has observed in the case of the *sea-urchin* Fol's "cône d'exsudation" *before* as well as after the approach of spermatozoa, up to the time when the elevation of the vitelline membrane and the expansion of the mucous layer have caused it to disappear; but he regards it (the cone) as the optical projection of the walls of the opening which constitutes the interruption in the continuity of the mucous layer. The "soft and plastic layer" of the vitellus cannot be compared to the envelope of the Amœba, since the spermatozoa after traversing the mucous layer meet here an impenetrable obstacle.

Giard (**'77**[b]) does not recognize the "necessity of employing spermatozoa in homœopathic doses," since those conditions are not realized in nature, nor does he understand how it is that Fol depicts *eleven* spermatozoa on a limited portion of the surface of an egg, if in his experiments he allows to each only three or four spermatozoa. From 10 to 15% of the eggs he himself has studied present pathological peculiarities, among which are *not* included cases of the formation of a *tetraster*, which he considers due to an ontogenetic abbreviation, not resulting in monsters, and comparable with Strasburger's observations on gymnosperms. Fol's statement that the *sea-urchins* present no hyaline protuberance is certainly not applicable to Psammechinus, where this protuberance is to be seen with the greatest ease. If Fol's view concerning the vitelline mem-

brane is accurate, the polar globules ought *always* to be found *outside* the membrane in all cases of normal fecundation ; they are, on the contrary, applied to the vitellus so that they are difficult of observation.

Fol (**'77**[f]) responds to Giard by saying that in the sea-urchins of the Mediterranean which he has studied there does not exist the "cône d'attraction" which *in the egg of Asterias is formed in front of the most advanced spermatozoön ;* not a single hyaline protuberance appears on the mature egg of these sea-urchins before fecundation.

The concurrent evidence of O. Hertwig's and his own studies shows that the polar globules are promptly detached from the ovule, not being retained by any membrane, and are lost in the ovary. Fol finds very small and pale corpuscles lodged inside the *outer* of the two vitelline membranes which exist in the sea-urchin. There are usually more than two, and as the globules described by Giard appear to correspond with these, he concludes that this author has not observed the true polar globules. Instead of traversing the supposed vitelline membrane by way of diffusion, as Giard thinks, the body of the spermatozoön penetrates, as such, the vitellus, a fact still demonstrable in his preparations.

Fol's (**'77**[g]) communication to the Swiss Society of Natural Sciences in August contains, beside what has already been given, some points which are not previously dwelt upon. In the starfishes there is only one vitelline membrane formed, but in the sea-urchins a second membrane is formed beneath the first, although it is not detached from the surface of the yolk until the moment of the first segmentation. In the third of the experiments here recounted, the eggs of the sea-urchin were fecundated by mixing them in sea-water with very dilute spermatic fluid, and then at once removed by a pipette to 2% acetic acid, followed successively by osmic acid and Beale's carmine. All these eggs have at one point of their surface a membrane raised up in the form of a watch-glass bulging in the middle and continuous at its margin with the limiting membrane of the yolk. At the centre of the region covered by this membrane the body of a spermatozoön is implanted in the surface of the yolk by its point. It lies in the direction of the radius of the egg, and has a cue. In eggs hardened a little later the body of the spermatozoön, recognizable by its shape and the color imparted by reagents, is sunk completely into the yolk so that its blunt end is "flush" with the surface. In place of the cue is a vesicle attached to the spermatozoön on the one hand and to the vitelline membrane on the other. The latter is now elevated from the yolk on all sides. A comparison with living eggs and those hardened simply in

osmic acid shows that the vesicle is the "cône d'exsudation" swollen by the action of the acetic acid.

In the case of abnormal fecundation, where two or three of the male asters have united with the female nucleus, the remaining male asters are very regularly placed at equal distances from each other and at about a third of the distance from the surface to the centre of the yolk. This proves (1.) the attraction of the male asters for the female nucleus up to the time of its saturation, and (2.) the mutual repulsion of the male asters, for otherwise their arrangement, irregular at their first appearance, would not subsequently become regular. Eggs that have received two spermatozoa have always been seen to form a *tetraster* instead of an amphiaster at the first segmentation. In certain cases of sea-urchins kept a short time in confinement, a large majority of the artificially fertilized eggs have exhibited the tetraster; almost all the larvæ were monsters. It is possible in certain vegetables, and even in certain animals, that the tetraster may not be a pathological phenomenon, but in the sea-urchin and starfish it is positively pathological as a rule, and it is doubtful if such an egg can produce a normal larva.

Fol (**'77**^{h}) has also published a reply to the criticisms of Pérez and Giard, which is more extended than any of the papers cited; but since it contains nothing essentially new, a review of it will be unnecessary.

Hatschek (**'77**^{a}, pp. 503–505, Taf. XXVIII. Fig. 1) finds within the pear-shaped egg membrane of Pedicellina (whether vitelline or secondary membrane is left unsettled) sometimes a small, sometimes a large number (50) of active spermatozoa. This is evidence of the existence of a micropyle. At the vegetative pole of an egg which had two polar globules there was seen a clear protoplasmic body, free from yolk granules, which, in the course of two or three minutes, became lost to vision by sinking into the yolk. Hatschek considers this a metamorphosed spermatozoön; but it seems probable that it should rather be compared with similar hitherto unexplained protoplasmic protuberances from the vegetative pole of the egg which usually occur after impregnation.*

A possible objection to this view exists in the fact that these protuberances are not always destitute of yolk granules, as Hatschek affirms of the body he has observed; but this may be subject to variation in different cases.

Hoffmann (**'77**, p. 19) has observed in the case of Malacobdella that the spermatozoa do not always penetrate the yolk with the head end, but often bore in with the tail end, and continue in activity for an hour

* Compare Whitman **'78**^{a}, pp. 21, 39, and O. Hertwig **'78**^{a}, Taf. XI. Fig. 4.

after fertilization. This account seems to need confirmation, for the greatest care must be exercised to insure the observer against the possibility of having before him eggs that are no longer in an active living condition. Hoffmann has observed clear protoplasmic elevations on the surface of the yolk, such as Bütschli has figured for Nephelis, but since the same phenomenon is seen on eggs that have certainly not been brought within the influence of spermatozoa, he thinks it cannot be that it has resulted from a metamorphosed spermatozoön. It may be considered as *certain*, he holds, that the penetration of the spermatozoa and the subsequent lively motion of the yolk granules induce the gradual disappearance and probably the complete elimination of the nucleus (germinative vesicle), *for* on artificially fecundated eggs so many spermatozoa are sometimes attached to the yolk as to put it in rotation, and in such cases the nucleus is ejected in its full size an hour after fertilization, whereas normally, when only a few spermatozoa are attached to the egg, the two small polar globules do not appear until two hours after fertilization. (!)

In the case of Clepsine, Hoffmann (**'77**[a], p. 34, Taf. III. Fig. 5) seems to have seen something of the asters of the male and female pronuclei. He has figured and described in a section of an egg prepared *several* hours after extrusion, the existence of two places in the coarsely granular yolk which are filled with a finely granular substance, radially arranged about the centre of each spot.

In Toxopneustes variegatus Selenka (**'78**) has observed the penetration of spermatozoa of which he gives this account. Usually only one spermatozoön succeeds after a long boring motion with its pointed head in passing through the jelly-like zone which envelopes the egg. As soon as it gets near the yolk it is suddenly enabled to swim rapidly and easily in all directions over the plasma mantle which envelops the yolk (see p. 458). The passage it has made through the zona remains open and is often traversed by both inward- and outward-going spermatozoa. The spermatozoön usually penetrates the yolk at the "Dotterhügel," and causes a distinct agitation in the surrounding parts by its boring motion. A tufted mass of clear substance at once collects around the head of the spermatozoön from the clear mantle of protoplasm which surrounds the egg. As it penetrates deeper into the yolk this tuft of protoplasm sinks with it; thus forming a depression from the middle of which the "tail," which soon becomes motionless, projects as a fine filament. When it has penetrated about a quarter of the way to the centre the automatic motion almost instantly ceases, and within half a

minute there is formed around the "head" the well-known stellate figure. In the course of a few minutes the "head" advances to the centre of the egg and remains there till the arrival of the egg nucleus. Meanwhile the rays increase in number and in length, and at the same time a "clear area," formed by the accumulation of protoplasm free from granules, makes its appearance at the centre of the system around the "head" of the spermatozoön. The "neck" of the latter gradually swells until it attains one third the diameter of the egg nucleus. The highly refractive tip of the spermatozoön is meantime thrown off and borne away by the ever-active yolk protoplasm; like the tail, it is apparently resorbed.

It always appeared as though a gentle amœboid motion of the egg nucleus began only at the moment when the rays surrounding the sperm nucleus had extended to it, as though it were thereby induced to begin its migration to the centre of the egg, along the course marked out by these protoplasmic rays. But in any event an automatic amœboid motion of the egg nucleus must be maintained. If it is granted that definite courses for the streaming protoplasm are present in the yolk, (of which, however, nothing is known with certainty,) it is not evident how the nucleus could be urged into the centre of the yolk by such currents of protoplasm, since the masses of the latter moving in centrifugal and in centripetal direction must be equal, and since the egg nucleus would therefore receive the same impetus in opposite directions.

A direct union of the two nuclei follows, and is accompanied by very active changes of form on the part of the egg nucleus, which sends out thick pseudopodia-like projections enveloping the "Spermakern," and then suddenly fuses with it.

As soon as the point of the head of the spermatozoön has penetrated the plasma mantle of the yolk there is raised up from the latter (within two minutes) a fine membrane, which pushes before it the zona and absorbs by diffusion the now fluid substance of the latter. In five minutes the membrane is far removed from the yolk, and the zone is no longer visible.

The spermatozoa may penetrate the yolk at any other place than the "Dotterhügel" without influencing the subsequent development, which also continues to go on for a time in a quite normal manner, when two, three, or even four spermatozoa at one time, or in quick succession, penetrate the yolk at the "Dotterhügel," or at different places on its surface. In this case each "head" acquires independently its radial figure. The author does not, in view of the observations of Fol and

Hertwig, place much confidence in this observation of normal development after such fecundation. A fusion of sperm nuclei is not to be seen; on the contrary they are mutually repelled by their astral rays.

Extensive studies on the nature of fecundation among plants lead STRASBURGER ('77) to a modification (p. 483) of his previously expressed views,* for he now holds that not all of the contents of the pollen tube are taken up by the egg *nucleus*, but that a part of it becomes directly mingled with the protoplasm of the egg. It now appears improbable to him that the portion of the fecundating substance which is destined for the egg nucleus can be appropriated by the latter in the amorphous condition in which it enters the egg, without having first assumed the form of a nucleus. This view is then generalized and sharply formulated (p. 508) as follows: "It is the equivalent parts of both cells which are united in fecundation." Support is afforded this view by the process of copulation in the "Gameten" of Acetabularia and Spirogyra, in the conifers, and especially in the case of those metasperms whose egg nucleus contains only a *single* nucleolus, and where the sperm nucleus in like manner embraces only a *single* nucleolus. For he finds that in such cases (e. g. Monotropa, pp. 488, 489, Taf. XXX. Figs. 127–129, 131–133, 135, 138) the new cell nucleus (male pronucleus) and the egg nucleus unite without the disappearance of their nucleoli, so that *two nucleoli*, which ultimately unite, are distinguishable for a considerable time within the conjugated nuclei. This is the more noticeable in the case of Monotropa from the fact that the male pronucleus and its nucleolus are constantly somewhat smaller than the corresponding egg nucleus and nucleolus. The division of the pollen cell shortly before fertilization, from which result a greater and a smaller ("vegetative") cell, is found by Strasburger to hold true with metasperms (p. 450) as well as with archisperms. With the formation of the pollen tube the nuclei of *both* migrate — the nucleus of the large cell foremost — usually into its tip (p. 456), where they take part in fecundation. But in the archisperms the "vegetative" cell is resorbed while the nucleus of the larger cell migrates to the tip of the pollen tube and there undergoes successive divisions, — two or more. The tip of the pollen tube is never broken through. Its protoplasmic contents are thought (pp. 483, 490) to pass both the membrane of the pollen tube and that of the embryo-sac, not in a diosmotic way, but directly, as a homogeneous viscid mass. The same force which has impelled the protoplasm during the growth of the tube toward its tip now causes it to advance in the direction of (i. e.

* See Strasburger '76, pp. 308, 309, and '76[a], p. 402.

into) the embryo-sac. The author thinks this assumption is supported by certain results obtained by Maxime Cornu (Comptes rendus de l'Acad. des Sciences, Paris, Tom. LXXXIV. p. 134). Of the two nuclei which are found in the tip of the pollen tube at the beginning of fertilization, the one in the rear (from the "vegetative" cell) is the first to disappear. For this reason Strasburger says it may be that it is the substance of the *other* nucleus which is more especially concerned in the act of fecundation (p. 487), its substance being preserved in a nuclear form up to the very instant of fecundation. The homogeneous condition here brought about just before fecundation by the disappearance of every nuclear structure makes the fecundation of phanerogams in a sense parallel with that of the higher cryptogams where the spermatozoid, though a "formed" structure, has no nuclear differentiation, but is a homogeneous band in which the nuclear substance is probably distributed uniformly. In plants where the copulating elements remain in an indifferent condition, i. e. indistinguishable from each other, there may be more than two such individual elements concerned in the act (e. g. Spirogyra, etc.). With a differentiation of the sexual products the possibility of this appears to cease.* Why in general only one spermatozoid is admitted to an egg, remains undecided. In the case of plants it may be owing to the extremely rapid production of a cellulose envelope, yet molecular processes of an altogether different character may in this case come into action.

The experiments of Fol and O. HERTWIG ('78) in the fecundation of starfish eggs are in many ways mutually confirmatory. In others their opinions differ. According to Hertwig eggs that are fertilized any time between the formation of the first maturation spindle and the completion of the egg nucleus afford evidence of the penetration of only a single spermatozoön; but there is this difference in the two cases. When the fertilization takes place at the earlier date, the male and female pronuclei attain equal size before their confluence, although the male pronucleus remains comparatively small and with little influence on the surrounding protoplasm up to the time the second polar globule is formed. On the other hand, when fertilization ensues only after the formation of the egg nucleus, the male aster grows rapidly in size, but the male pronucleus remains a much smaller structure than the female. Hertwig explains this by supposing that in the latter case the female pronucleus has be-

* I do not understand how the observations of the author necessarily exclude the nucleus of the "vegetative" pollen-cell from participation in the act of fecundation; and if not excluded, the above sentence does not seem entirely justified.

come possessed of all the available nuclear fluid of the yolk; that in the former they imbibe this fluid to the same extent. These two experimental cases he maintains will serve to explain differences which exist in different groups of animals. In these cases both of which are considered normal, the yolk promptly retracts from the vitelline membrane after impregnation, and one often sees a minute bridge of protoplasm connecting the surface of the yolk with the membrane near the vegetative pole, — probably the place of penetration of the spermatozoön. If the impregnation is undertaken either before or after the epochs mentioned, abnormal phenomena are the result. The yolk withdraws from the vitelline membrane not at all, or only slowly. A number of isolated stellar figures appear in the cortical portion of the yolk, and remain limited in extent. If the fertilization is effected during the metamorphosis of the germinative vesicle, the development proceeds normally to the end of the formation of the polar globules, but not further; if it is effected too late (six hours after exclusion) two or three of the male asters may approach close to the egg nucleus, and the latter sometimes takes an oval form. Normal segmentation does not follow in either of these cases.

Hertwig disagrees with Fol as to the formation of a vitelline membrane at the time the spermatozoön penetrates the egg. The vitelline membrane, he claims, already existed, and the interval between it and the yolk is brought about by the contraction of the latter, which is accompanied by the pressing out of the perivitelline liquid already observed by the older naturalists.

The results which Calberla ('78) has reached in his recent paper on the fecundation of the eggs of Petromyzon Planeri he has himself condensed into the following form (p. 477). A single spermatozoön enters through the outer micropyle into the space between the egg membrane and the yolk. This space is filled with protoplasm free from yolk granules. The contact of the spermatozoön with this sets in activity a stimulus which results in a slight contraction — an amœboid motion — of the yolk, which makes itself apparent in a separation of this clear layer from the egg membrane in the vicinity of the micropyle. This partial separation of the egg membrane from the yolk now makes possible an influx of water into the perivitelline space thus formed. Such an inflowing of water was previously prevented by the pores of the egg membrane being sealed up by the peripheral layer of clear protoplasm. By this influx of water the egg membrane is widely separated from the yolk. Authors who ascribe the existence of a great space between the vitellus and membrane in lower vertebrates to an extensive contraction

of the former, are in error; the yolk suffers only a minimum contraction, and that occurs in the vicinity of the micropyle. This he has demonstrated also in batrachians and bony fishes. Inasmuch as the peripheral layer of protoplasm adheres in places to the egg membrane, the protoplasm is drawn out, by the invasion of the water, into fine threads connecting the surface of the yolk to the membrane. These ultimately rupture, one end going to form drops on the inner surface of the membrane, the other, elevations at the surface of the yolk. At the micropyle a much thicker cord of protoplasm — the guiding cord of the spermatozoön (Leitband) — has the same connections. It is through this "Leitband" of protoplasm that the head of the spermatozoön penetrates to the inner micropyle,* and thence into the sperm passage, and thus reaches the egg nucleus. Calberla furnishes only very unsatisfactory evidence that the head of the spermatozoön actually advances to the egg nucleus. In sections of hardened eggs he has sometimes seen in the sperm passage an indistinct elongated structure, which he would refer to the head of the spermatozoön. The actual conjugation of two nuclear structures cannot be claimed to have been observed. While a part of the "Mittelstück" of the spermatozoön may enter the sperm passage, its tail, he asserts, does not enter the egg but remains to plug up the micropyle, and thus prevent the passage of other spermatozoa.

With the further removal of the egg membrane from the yolk this cord of protoplasm (Leitband) is severed; its peripheral end forming a great drop on the inner surface of the membrane at the "outer micropyle"; its central end forming a "Dottertropfen" in front of the inner micropyle. Usually this "Dottertropfen" is drawn for a short time within the yolk, only to appear again in consequence of a contractile process within the egg which is connected with a stellar arrangement of the yolk granules.

Concerning the egg nucleus during the penetration of the spermatozoön, Calberla (p. 465) says it is altered, it becomes indistinct, but it does not lose its morphological identity.† It is during this loss of distinct contour that the yolk granules arrange themselves in rays around the disappearing egg nucleus. Subsequently one sees in its place a new nucleus, with sharp contour, which he identifies with Hertwig's "*Furchungs*kern." As soon as this segmentation nucleus is formed, the contraction of the yolk ceases and the "Dottertropfen" retires into the

* Compare the review (p. 456) of the maturation phenomena as described by Calberla for Petromyzon.

† "Jedoch nicht zu Grunde geht."

yolk of the sperm passage. This marks the termination of the act of fecundation.

If Calberla's explanation of the reappearance of the "Dottertropfen" is correct, this structure affords a very important index, — easily recognized in the living egg, — not only to the *time* at which the contraction takes place, but also to the *energy* with which it acts at any given instant. Calberla has further described this process of contraction as an arrangement of the yolk granules concentric to the egg nucleus, which makes its appearance at once upon the accomplishment of the nuclear copulation. As the stages of copulation can hardly be said to have been satisfactorily observed, it follows that the most which can be justly claimed is that this stellate arrangement is to be observed about the time of the supposed copulation. If Calberla is right in the interpretation of the elongated body "*Spk*(?)" indicated in his Fig. 8 as a sperm nucleus, then it is clear the radial arrangement *precedes* nuclear copulation and has the *egg* nucleus for its centre, as he himself in one place clearly indicates.

Even if fertilization does not take place, the egg membrane after a time — twelve hours if the eggs are maintained in cold running water (+8° to +10° C.), sooner if in warmer water — is elevated from the yolk. But whereas in the former case this elevation began *around* the micropyle and ensued next *at* the micropyle and only secondarily, as it were, over the rest of the yolk, in the latter case it takes place slowly and uniformly over the whole surface and without definite relation to the micropyle. Moreover, in the latter case neither threads of protoplasm nor a "Leitband" are formed. Although a "Dottertropfen" appears at the inner micropyle, it is not withdrawn into the yolk and subsequently made to protrude, but ultimately ruptures and is soon followed by the disintegration of the whole yolk. From the moment of the first appearance of the elevation of the egg membrane, even if over only a limited area, the egg becomes incapable of fecundation. How far a possible change in the condition of the superficial clear layer of protoplasm might interfere with the penetration of spermatozoa could not be established, as the latter were never seen in these cases to reach the perivitelline space formed by the elevation of the egg membrane. The existence of a "Dottertropfen" in unfertilized eggs Calberla endeavors to explain by assuming that the inflowing *water* exercises on the yolk a stimulating influence which induces a slight contraction and consequent protrusion of part of the contents of the sperm passage.

In a short supplement to his paper Calberla compares this "Leitband" with the conical elevation observed by Fol in the case of Asteracanthion,

and by way of inference makes its existence due to an attractive influence exercised, at a distance (?), upon the yolk by the spermatozoön.

He thinks that there is no vitelline membrane formed in the case of Petromyzon at the time of fecundation, such as Fol has described for Echinodermata.

It remains to add that the phenomena of fecundation transpire more rapidly the longer the egg has been removed from the animal, provided it has not meantime suffered the change described above as occurring at the expiration of about twelve hours.

BALFOUR (**'78**[a]) has given a short synopsis of fecundation as observed by Fol, O. Hertwig, Selenka, Giard, and Calberla. He suggests that the pathological symptoms shown in the embryos reared by Fol and Hertwig may be due to an imperfection of the eggs, induced by a delay in impregnation, rather than to the entrance of more than a single spermatozoön.

In his paper on "Befruchtung," etc., VON JHERING (**'78**) treats, for the most part in a purely objective manner, of the recent discoveries in maturation and fecundation of animal ova. He emphasizes (p. 121) the fact that no morphological difference exists between nucleus and *pro*-nucleus. The acceptance of the idea of a pronucleus, in view of the parthenogenetic development of the starfish, is only possible with the reservation that the female pronucleus does not necessarily need the accession of a male pronucleus in order to become a segmentation nucleus.

The final paper by SELENKA (**'78**[a]) on Toxopneustes variegatus, besides contributing minor additional details concerning fecundation, contains essential modifications of the preliminary notice. In the first place, the union of the two pronuclei is not accomplished quite so promptly as at first supposed. The first contact only results in a welding which lasts about fifteen minutes, during which the male pronucleus grows until it reaches the size of the female pronucleus. During all this time, the boundary between the two remains distinguishable. It seems to have been previously mistaken by Selenka for the beginning of the spindle differentiation. Finally, however, the limit between the two pronuclei entirely disappears.

He corroborates Auerbach's observations of a rotation of the joined pronuclei so far as to say that the long axis of the elliptical conjugation-nucleus soon becomes oblique to the radius along which the spermatozoön penetrated. Whether this takes place only in the case where the course of the spermatozoön is predetermined by the existence of a

micropyle, or "Dotterhügel," cannot yet be decided. He says he has sometimes seen its rotation through 90° of arc, and adds, that "*therefore,* since the spermatozoön as a rule penetrates at the 'Dotterhügel,' the first plane of division passes through that point (Dotterhügel). But inasmuch as the latter also indicates the place where the directive corpuscles emerged, one may also say that the first segmentation plane generally coincides with the radius which is determined by the long axis of the nuclear spindle or by the course along which the directive corpuscles emerge." "But," he adds, "it is not to be forgotten that the spermatic *element may also penetrate at other places than at the Dotterhügel,* and that then, not the 'Mikropylenhügel,' but *the radius along which the spermatozoön has penetrated would determine the direction of the first cleavage plane.*"

Resting on this argument, Selenka claims that it is not right to consider that part of the yolk where the directive corpuscles emerge the formative pole, and therefore that the name polar globules is not suitable for the directive cells of Toxopneustes.

I believe that Selenka is in error in saying that the plane of cleavage is determined by the line along which the spermatazoön penetrates, and think the protuberance called Dotterhügel may be the source of the difficulty. This the author has defined as the elevation left at the place where the directive corpuscles escaped. He has doubtless observed cases in which the first cleavage plane did not pass through this elevation, and hence concludes there is not a constant relation between the position of this plane and the place where the polar globules emerge. Unless the identity of the Dotterhügel and this place of emergence are indisputably established, his conclusions will not necessarily follow from his observations.

I do not see that that there is any conclusive evidence that the "Dotterhügel" may not be some other protuberance of the yolk than that which was left behind by the polar globules; for example, such an elevation as Giard has called the second cumulus, and which Fol has affirmed to be a projection of the yolk into a place of the oölemma corresponding to the point of the egg's ovarian attachment. That this may really be the case here seems none the less probable from the remark which Selenka (p. 6) himself makes, to the effect that "the spermatozoa seem to penetrate the gelatinous mantle preferably in the immediate vicinity of the 'Dotterhügel,' and that this is apparently a more practicable passage, — it may be on account of the emergence of the polar globules at this point, it may be because it is at

the same time *the place where the pedicellate egg, up to a short time before its liberation, was connected with the ovarian wall*, and that therefore the mantle is here softer." The fact that the absence of a membrane allows the polar globules to lose entirely their connection with the yolk, makes such a mistaken identification as I have suggested extremely easy, or at least would make the proof of the accuracy of the identification attainable only by continuous observation of the same egg. If Selenka's grounds for disconnecting the polar globules and the position of the first cleavage plane are insufficient, those for connecting the position of the plane with that of the radius along which the spermatozoön penetrates are limited to the statement of the fact, and therefore lie beyond the reach of criticism.*

If those of Selenka's conclusions which I have criticised should prove to be untenable, the objections which he urges against the use of "formative pole" and "polar globules" in Toxopneustes would be no longer valid.

While Kupffer und Benecke ('**78**) confirm the most interesting point of Calberla's observations on Petromyzon, — the penetration of a spermatozoön into the yolk, — they differ in many points of importance. The micropyle is not always situated at the summit of the watch-glass-shaped portion of the egg membrane. Only the *inner* layer of the egg membrane is provided with pore canals, not both layers, as Calberla claims. The cap of clear protoplasm which lies immediately under the watch-glass area of the membrane is not continued as a thinner layer around the whole yolk, but has about the same extent as does the watch-glass elevation. Only those spermatozoa which attain the hyaline dome (A. Müller's "Flocke") surmounting the watch-glass segment of the egg membrane are of concern in the act of fertilization. All such at once assume a direction radial to the "watch-glass," and as soon as the first has reached this dome the yolk begins to withdraw from the egg membrane, leaving an annular space corresponding with the rim of the watch-glass. The contraction of the yolk therefore results from the influence of the spermatozoa *at a distance.* This retraction is more lively if several spermatozoa enter the dome instead of one. The further retrac-

* In what sense the spermatozoön radius is held to be "determining" can possibly be *inferred* from an examination of his figures. From Figs. 16 and 17 it appears that the first cleavage plane (if perpendicular to the long axis of the nucleus) neither coincides with nor is perpendicular to this radius. Therefore, the only conclusion that seems possible is that the position of the segmentation plane is held to be dependent on two factors, — one the direction of the spermatozoön radius, and the other the extent of the angular rotation of the fusing pronuclei.

tion of the yolk results in the formation of the protoplasmic filaments and the "Leitband"; the latter, however, is not constant, nor does it, when present, always serve to guide a spermatazoön; on the contrary, a spermatozoön may penetrate the membrane at any point of the watch-glass, even near its margin. The term "Axenstrang" is substituted for "Leitband," as the function implied in the latter word cannot be proved to exist. The foremost or "preferred spermatozoön" enters the yolk *in toto*, not leaving any part of its tail behind, as Calberla claims; but as soon as the head has entered the egg membrane the activity of the spermatozoön seems to cease, the tail becomes stiff, and the whole is *drawn* onward. The head part becomes more and more elongated, as it approaches the yolk. All *other* spermatozoa which penetrate the "watch-glass" a greater or less distance do not thus cease their activity, and in addition to the motion of the tail there seems to be an amœboid change in the outline of the head; a wave-like motion is observed to pass forward along the head to its free end. If the head has advanced near to the inner surface of the membrane, it sends forward a fine filament, like a pseudopod, which traverses the interval; waves advance along this filament, which becomes swollen at the end, and finally the swollen part becomes detached as a clear vesicle in the perivitelline space.

The retraction of the yolk is due to an actual contraction of its substance rather than to an invasion of water as claimed by Calberla. The contraction is of such a nature that at one time the half of the yolk occupying the active end of the egg has the form of a truncate right cone, while the other half retains its ellipsoidal outline. At this time, about three minutes after the union of sperm and eggs, a mass of clear protoplasm (Calberla's Dottertropfen) rises up in the centre of the truncate surface, enlarges and extends till, in about three minutes, it reaches the inner surface of the membrane, which, by its amœboid motion, "it licks off," and then with many changes of form it retires. By this process it incorporates with it the vesicles which Calberla called "Randtropfen," but which are not all of them the retracted ends of the protoplasmic filaments left by the retiring yolk, for a part at least were formed by the *spermatozoa* in the way above indicated. This "Dottertropfen," occasionally at least, also envelops unaltered spermatozoa which have effected their entrance into the perivitelline space. It is not simply the again protruded "Leitband," for it is occasionally absent, and when present is of greater volume than the latter. This protoplasmic projection exercises an active supplemental *rôle* in fecundation. While to one

spermatozoön falls pre-eminently the function of fecundation, other spermatozoa may participate in the act through the active intervention of this protuberance of hyaline protoplasm.

To explain these phenomena the authors assume that a body (Eikern) in the yolk, which at the approach of spermatozoa enters upon a state of activity, exercises an attraction both on the protoplasm of the yolk and on the spermatozoa, which diminishes with the distance, but increases with the mass, and that this body is movable in the yolk. The radially arranged spermatozoa at the surface of the "watch-glass" give the impetus to a change in the nearest protoplasm of the egg, by means of which the [egg] nucleus is formed. The first effect is the detachment of the protoplasm from the "watch-glass," and the attraction of that spermatozoön which meets with the least resistance. Since the attraction of yolk and nucleus is to be considered as mutual, and as proportional to the mass, and since the greater part of the egg lies on the side of the eccentric nucleus toward the passive pole, it follows that the yolk will communicate to the nucleus a motion toward its (yolk's) centre. The nucleus thus becomes further removed from the "watch-glass," and its attractive influence on spermatozoa diminishes. Therefore any spermatozoön which might chance to follow the same (microyplar) radius as the first one would be less strongly attracted, and thus may be explained why as a rule only one spermatozoön passes the membrane through the micropyle.

From the nature of their fertilization it was not possible for Whitman (**'78**[a])* to observe the penetration of spermatozoa into the eggs of Clepsine. A nuclear body, which he from analogy concludes is the male pronucleus, makes its appearance usually about the time the second polar globule is formed. It has also been detected before the formation of the first polar globule. It is found near the centre of the egg before the female pronucleus has receded much from the oral pole. The nature of these pronuclei will be discussed farther on. During the formation, or at least during the migration and conjugation of the pronuclei, remarkable changes occur at the poles of the egg, which previous observers had seen only from the surface and designated as "polar rings." By means of sections Whitman was enabled to study the internal changes which the substance of these rings undergoes.

About fifteen minutes after the second polar globule is formed a transparent fluid substance begins to collect in a shallow groove which encircles the oral (animal) pole, thus forming the first polar ring. At

* See also Whitman **'79**.

first feebly expressed, this ring soon becomes well defined and has on either margin a border of yolk substance that is destitute of yolk spheres, but densely packed with fine granules, and appears whitish in reflected light. This ring deepens and at the same time approaches the pole so that a central cup-shaped mass of the yolk is nearly cut off from the main mass, having only a slender stalk of connection, like the stem of a goblet. At the same time the outer or equatorial margin of the ring becomes denticulate, and its substance stretches out towards the equator of the egg in the form of rays, — the "*ring rays*." About ten minutes after the appearance of the first ring a second one appears at the aboral (vegetative) pole of the egg, but in narrowing upon the enclosed space it does not dip so deeply into the yolk, and ultimately forms a superficial disk with thickened margins. This also sends out "ring rays." At the approach of cleavage the ring at the oral pole is made to assume the shape of a crescent by the movement of the cup-shaped mass of yolk toward that side of the ring which is nearest the plane of the coming cleavage. Both sets of ring rays become more and more feeble at the approach of segmentation.

On sections it is seen that the whitish substance which appears on the borders of the rings forms a continuous layer underlying them. After they have assumed, the oral one the shape of a compact, well-defined ring with nearly circular section, — the aboral one the shape of an oblate spheroid, — the "whitish" substance underlying and more or less surrounding them is seen to plunge deeply into the yolk at about the time the first cleavage amphiaster is forming. The substance of the rings takes the same course toward the cleavage nucleus. In osmic acid and carmine preparations the ring substance behaves in the same manner as the substance of the "nucleus"; it is therefore "probably nuclear matter, or something very analogous." The rings "possibly contribute some elements to the nucleus, which may either induce or stimulate the molecular changes, which result in the formation of the *primary cleavage amphiaster*."

The interpretation which Whitman gives the small sharply defined bodies occupying the centre of what he regards as the pronuclei is, as he himself fully understood, radically at variance with the more generally accepted view. Most observers have considered the structures in question, even though two or perhaps several in number, as the pronuclei themselves, and the surrounding ill-defined plasmic substance as a specially differentiated, or at least segregated, portion of the *yolk* protoplasm. Whitman, on the other hand, holds this homogeneous

plasm ("nucleoplasm") to be, not yolk plasm, but the pronucleus itself, and the bodies embraced in it to be *pronucleoli*, and by means of this interpretation (which is of course extended to the subsequent generations of nuclei and their nucleoli) is enabled to avoid the contradictory position in which Bütschli and others find themselves when they endeavor to explain the existence of several nuclei in a cell without thereby interfering with the essential character of the cell as a *uninuclear* structure.

It is to be remarked in the beginning, that Whitman saw only traces of a nuclear plate in the archiamphiasters, and these were so uncertain in character that they were entirely omitted from his drawings. This, I think, will serve to explain why he has been less successful in tracing the origin of the female pronucleus than some other observers whose more favorable objects have proved very instructive on this point.

Immediately after the appearance of the second polar globule a circular space directly below the latter, which appears in fresh eggs as a pellucid spot, is found in eggs treated with osmic acid and carmine to be filled with a very fine granular substance which has the lead-gray tinge and the feeble staining capacity characteristic of the germinal vesicle when similarly treated. This is the remnant of the archiamphiaster, and is called the *female pronucleus*. It is without membrane, perfectly homogeneous, and forms the centre of a radial system. On the inner (or deeper) side of the female pronucleus there are subsequently to be seen two small highly refractive corpuscles in close apposition, and together 10 μ in diameter. These are sharply defined, homogeneous, and more deeply colored than the nucleoplasm. They are female *pronucleoli*. Whitman gives no positive information as to the origin of these pronucleoli, whether they have a genetic connection with definite parts of the amphiaster or arise as new structures within the nucleoplasm. The male nucleus, which comes into view near the centre about this time, presents the same appearance, with, however, only one pronucleolus. Both pronuclei are surrounded with radial lines, and their longest axes lie in the main axis of the egg. In later stages only one nucleus is found, with its main axis oblique to that of the egg, but embracing both the male and female pronucleoli. The radial lines are fainter. Then this primary cleavage nucleus comes to lie a little eccentrically towards the oral pole, and its axis is at right angles to that of the egg. Its substance (nucleoplasm) is more strongly colored near the pronucleoli than at the periphery. Later the pronucleoli have become larger,

and are closely applied to each other. They are still sharply outlined, but only slightly stained with carmine.

The cleavage nucleus becomes more elongate, and, to judge from his Fig. 71, more sharply defined; but the pronucleoli do not become fused until the "nucleus" [central area] has assumed the spindle shape, nor even until the first cleavage spindle begins to form. Whitman, it is true, does not figure any stage in which the bodies in question remain intact after the cleavage spindle begins to appear; but I wish to call attention to the fact that he says (p. 24) such is the case, for it seems to me an important point in helping to prove the identity between the structures which Whitman calls *pronucleoli* and those which I have called the *pronuclei*. I do not understand how this late coalescence of the bodies in question corroborates the view that they are nucleoli rather than nuclei. I can see *a priori* no reason why either a dissolution or a coalescence of nucleolar structures should be delayed beyond the time when a like fate overtakes the nuclei; on the contrary, evidence is not wanting that in the metamorphosis of the germinative vesicle, where there will be no ground for disagreement as to the *nuclear* nature of the vesicle, the nucleolar elements are often the first to undergo radical change. Out of numerous cases I will cite only O. Hertwig (**'77**[a], pp. 277, 278) for Pterotrachea. The case of the germinal vesicle, it is true, is not one in which we have to do with the *fusion* of similar elements, yet when we consider that the metamorphosis in both cases is one which leads directly to the formation of the spindle, I think the justice of the comparison will not be denied. In Whitman's opinion, "the size, structure, chemical behavior, and destiny of these bodies" favor his interpretation.

It is of course important to ascertain at first if the bodies in question are really the same in the case of Clepsine and Limax. There appears little or no room to doubt this identity, notwithstanding their rather striking differences of size, since, on the one hand, Whitman identifies respectively his pronucleus and pronucleolus with the "Strahlensysteme" * and the minute corpuscles embraced in the same, as described for Nephelis by Bütschli and Hertwig; and since, on the other hand, the questionable structure in Limax, though ultimately far from minute,

* To assume that the *radial systems* are the nuclear structures (the pronuclei in this case) seems to lead one into the unfortunate position of being compelled to identify the whole, or nearly the whole, protoplasm with the nucleus, for the radial systems at one time or another stretch through the entire yolk; therefore I have come to the conclusion, which is supported by what is said elsewhere (Whitman, pp. 21, 25), that the author means to designate only the clear central *areas* of the radial systems as nuclear structures.

undoubtedly arises directly from the elements of the nuclear plate, and therefore unquestionably corresponds with the "minute corpuscles" in Nephelis, which have a like origin. Furthermore, the behavior of these structures in both snail and worm at the time the first cleavage spindle is forming, as already noted, is significant of their identity. But if they are in reality the same, then some of the arguments advanced by Whitman will lose in significance when applied to Limax. Size certainly cannot be claimed to indicate their nucleolar nature in Limax, nor will it be at all satisfactory to attribute their great dimensions to the action of such reagents as Bütschli used (acetic acid), since osmic acid confirms the substantial accuracy of the observations in this respect. It remains, however, none the less interesting and important to ascertain the cause of the excessive minuteness which these bodies continue to exhibit in Clepsine. I am inclined to think that a causal connection may ultimately be discovered between the diminutive size of these bodies and the segregation of a part of the nuclear substance to form the remarkable polar rings, which, I believe, are not as yet known to exist in other eggs. It is noticeable that the polar globules share this diminutive condition with the corpuscles in question.

The objections to Bütschli's studies, raised on the strength of Hertwig's observation that acetic acid does not afford so reliable results as does osmic acid, are in part valid, as an examination of the figures I have given of Limax will show; but a veritable membrane will hardly be claimed as a *necessary* part of a nucleus, any more than of a nucleolus. Further, the use of chromic and osmic acids clearly shows in the case of Limax (Figs. 52, 68, 70, 72) that these bodies are not perfectly homogeneous, but contain conspicuous structures, not at all to be confounded with vacuoles, and the reticulum which is characteristic of nuclei. The evidence of the acetic-acid specimens is in this point, then, substantially corroborated by the use of other reagents.

The testimony of most recent observers as regards the growth of these bodies at the expense of the surrounding clear substance (central area) is so uniformly the same, that it does not seem necessary to dwell upon this point. It appears to me that an even greater obstacle to Whitman's interpretation is afforded by the *male* pronucleus of Limax, which grows in size at the expense of the surrounding protoplasm without the intermediation of any radial system or *central area.* One is compelled to ask, If the body in question is a male pronucleolus, what evidence have we in Limax of the existence of a male pronucleus? Yet this structure is so entirely similar to what I have called the female

pronucleus that their morphological equivalency cannot for a moment be doubted. For Clepsine, however, Whitman speaks with the greatest positiveness that "the central area does not disappear, nor even diminish in size," though it is granted that the corpuscles increase a little in their dimensions.

The chemical behavior of these corpuscles, even in the case of Clepsine, does not appear to me to be inconsistent with their nuclear nature; at least, that they are "more deeply colored than the nucleoplasm" is entirely consistent with an interpretation which makes of the "nucleoplasm" yolk-protoplasm, and of the corpuscles, nuclei, since, as is known, nuclei stain more vigorously than the surrounding protoplasm of the yolk.

The destiny of these bodies is to coalesce and then to participate in the formation of the spindle. But their smallness in *Clepsine* seems to preclude the idea of the spindle being formed exclusively, or even largely, at their expense, whereas the size and elongation of the "nucleoplasmic" area is such as to produce a conviction that the spindle is formed by the metamorphosis of the latter. Add to this the fact observed by Whitman, that this nucleoplasm exhibits the same reaction under treatment with osmic acid as the contents of the germinal vesicle, and we have the strongest points that can be made in favor of Whitman's interpretation. I shall not attempt to deny that both "central area" and spindle in Clepsine are largely due to a metamorphosis of this segregated plasm which Whitman calls "nucleoplasm"; on the contrary, I think we have good reason to believe that the same is true, though to a less extent, in the case of Limax (compare Fig. 85); that is to say, that in Limax also the two central areas arise outside of (though not necessarily quite independently of) the two unfused bodies which Whitman calls pronucleoli, and that they and the spindle owe their origin in part to protoplasm which lies quite beyond the limit of those two bodies. The only essential difference in the two cases will be this: that, while in Limax *all* the nuclear substance (nucleoplasm) is ultimately segregated to form a large nuclear structure, in Clepsine the other events of the metamorphosis overtake, as it were, this segregating process while enough nuclear substance still remains diffused in the neighboring protoplasm of the yolk to give the observed reactions under the influence of osmic acid.* But admitting the possibility of the exist-

* I wish to emphasize at this point the observation previously quoted from Whitman, — that "the nucleoplasm is more strongly colored in the centre, around the pronucleolar bodies, than at the edges," — for, in my opinion, this is evidence that his so-called nucleus is not uniformly nuclear substance (nucleoplasm), but only proto-

ence of a potentially or prospectively nuclear substance outside the corpuscles in question, does not involve the necessity of considering such unsegregated substance to be a veritable nucleus.

However unsatisfactory this attempt to harmonize these two cases may appear, I cannot hesitate to adopt it, if — as seems to be the case at present — the only other alternative is to hold the bodies under consideration to be in the case of Limax *pronucleoli.*

O. Hertwig ('**78**[a]) has established in Mitrocoma the existence of a vacuole much smaller than that which is derived from the inner half of the second maturation spindle, and considers it to be the sperm nucleus, even though no radial structure was discovered in the surrounding protoplasm. He thinks this may be due to the difficulty of recognizing such a radial structure when the protoplasm is homogeneous, as it is here. From the evidence thus far gained in Limax it would seem that in other cases it might not be possible to distinguish the stellate structure, and that, too, where Hertwig's explanation would be insufficient. These two nuclear vacuoles, he continues, become mutually flattened, and in the living egg suddenly cease to be visible, but in their place may be found, after treatment with acetic acid, the customary spindle and amphiaster.

In artificially fertilized eggs of Sagitta the yolk, before the first polar globule is formed, withdraws somewhat from the vitelline membrane, and a faint radiation may be detected at the vegetative pole. This remains, as in the starfish, inconspicuous until the second polar globule begins to be formed, when it increases in extent, and migrates toward the centre of the yolk. It appears, says Hertwig, as though the plasma when ruled by the process of division going on at the animal pole, could not respond to the stimulus of the male nucleus in so liberal a manner as later.

Interesting features of the conjugation of the two pronuclei in Sagitta are, that as they approach each other they become pointed, and that the pointed ends become applied to each other and assume a darker aspect (see Hertwig's Taf. X. Fig. 18), as though the more compact elements were collected there, and the more fluid had receded to the opposite ends of the pronuclei. As far as relates to the altered form

plasm, in which the amount of infused nuclear substance at any given point is proportional to its nearness to the central corpuscle. The *gradual* transition in the nature of this "nucleoplasm" till it is no longer distinguishable from the surrounding yolk protoplasm, appears to me an important objection to considering it the nucleus.

this is very similar to what I have observed on hardened eggs of Limax, and since Hertwig's observations were made on *living* eggs there can be little doubt that the condition I have represented in Fig. 68 is entirely conformable to that which existed before the eggs were hardened. The figure which Hertwig gives represents these structures as being more *angular* than those I have figured, and I have detected no appreciable difference in density between the narrower and broader ends. On the other hand, Hertwig has figured the outlines as of equal distinctness throughout, even when the pronuclei are in close proximity to each other. For Limax I must repeat that, while the outline of each pronucleus is not interrupted or effaced, it is much less boldly expressed at and near its more pointed extremity. I doubt if this indicates a difference in the composition of the two extremities of the nucleus. May it not be that the density of the *surrounding protoplasm* changes with its distance from the central point of attraction, in such a manner that it more closely approaches the refringency of the pronucleus at the smaller extremity of the latter than it does at its more obtuse end? Sagitta and Limax afford the only cases, so far as I recall, where this peculiar shape of the pronuclei has been observed.

Hertwig's observations on the formation and copulation of the pronuclei in mollusks afford several interesting points of comparison with Limax. Thus, in the case of Mytilus, he says that they melt together, and then become indistinct. In a pteropod (Tiedemannia), soon after the first appearance of the two pronuclei, there was seen emerging from the male pronucleus, in the part of the yolk not obscured by yolk granules, a filament which followed when this pronucleus advanced to meet the female pronucleus. Meantime the clear protoplasm increased in extent about the animal pole of the egg, and thus was brought to view successively more and more of the filament, which remained visible till the time of the first segmentation. Hertwig interprets this as being the filament of a spermatazoön, which penetrates at the vegetative pole, and remains at first concealed by the yolk granules of that portion of the egg. The head of the spermatazoön (i. e. the nucleus of the spermatic cell) gives rise to the sperm-vacuole, and the filament remains to be dissolved in the yolk during and *after* the first segmentation. The pronuclei in this case reach dimensions proportionate to the same structures in Limax. By the time they have attained this relatively great size, a large number of nucleoli have been formed (ausgeschieden) in them.

The next change which overtakes the nuclei does not seem to be the

melting together which is so generally observed to ensue. Hertwig says the nucleoli suffer disintegration into clusters of small granules, which collect on both sides of the conjugation surface; then two dull stellar systems make their appearance at two diametrically opposite points in this plane of contact; next, the contour of the two vacuoles suddenly disappears, the nuclear fluid apparently mingling with the surrounding protoplasm.

This agrees with what I have shown to take place in Limax, so far as concerns the origin of the amphiaster of the first cleavage sphere *before* the actual fusion of the pronuclei. Hertwig does not seem to have observed any want of synchronism in the appearance of the two asters, and moreover clearly places them on the *boundary* of the nucleus and the protoplasm, just as Fol and many others have done. Without giving particular prominence to the fact, Hertwig makes the disintegration of the pronuncleoli *precede* the appearance of the asters. That I consider to be a point of cardinal importance, and one which, in view of the contrary results I have obtained, and in view of the fact that Hertwig may not have observed the very first indications of an existing aster, should hereafter demand especial attention. Where do the first signs of the approaching segmentation make their appearance, — in the nucleus, in the yolk, or at the boundary of the two? The definite answer to this question will, I believe, advance us a step in the appreciation of the mutual relations of nucleus and protoplasm.

According to Hertwig, these changes occur in both Phyllirrhoë and Pterotrachea in the same manner as in the pteropods, save that in the latter genus the pronuclei secrete each only a *single* nucleolus. When their contours disappear, the pronuclei contain, in his opinion, *only* nuclear fluid (Kernsaft).

Blanchard (**'78**, pp. 754 – 758) summarizes the results of the recent studies on fecundation which he has reviewed, and expresses his belief that the homogeneous clear mass, at the expense of which the male pronucleus grows, is not vitelline protoplasm, but rather that part of the germinative vesicle which is dissolved in the vitellus at the moment when the directive (first maturation) spindle is formed. He admits that there is no direct evidence to prove this hypothesis: it simply seems to him more rational than the other supposition.

C. THEORETICAL CONSIDERATIONS AND GENERAL CONCLUSIONS.

Promorphology of the Ovum. — In the earlier stages of its growth from an indifferent cell, the ovum remains for a time in the condition of an *homaxial* body.* The polyhedral form, which so often obtains, is not of fundamental significance; it is simply the temporal phase, which its surroundings mechanically impose upon the ovum. This is clearly shown by the spherical form which such ova usually assume as soon as they are set free. The homaxial condition of the ovum is evinced by the central position of its nucleus, the germinative vesicle, as well as by the form of the protoplasm which constitutes its principal bulk. Whether granular or more homogeneous, the latter exhibits in every radius the same conditions.

Ultimately, however, one of its axes undergoes important changes, and thus becomes differentiated from the remaining axes; these still continue in a state of mutual equality. Such may be called the *monaxial* condition. Although the differentiation of this axis is manifest in a variety of ways, and perhaps occurs at different stages of development in different animals, there are still many reasons for believing that *this differentiated axis is homologous throughout the Metazoa.* It may properly be called the *primitive axis.* It is that — to mention a single one of its characteristics — in which the maturation spindle lies just prior to the formation of the polar globule. While the spindle may possibly furnish in some cases the first readily appreciable indication of such a condition, it is probably only one of the evidences of a *pre-existing* monaxial state.

Since the differentiation of the primitive axis usually appears to be coupled with a specialization of one of its poles, it remains at present doubtful if a *haplopolar* monaxial stage intervenes between the homaxial and the *diplopolar* monaxial conditions. I have, however, already (p. 186) called attention to a condition of the egg in the development of Limax which seems to realize the requirements of such a stage. The poles of

* Even in those cases (Vertebrata) where the permanent ova are formed by a confluence of several primitive ova, the homaxial condition appears to be only temporarily obscured, and is none the less an essential phase in the growth of the permanent ovum. See Goette **'75** and Balfour **'78**[b].

the primitive axis are often so unlike, that the differences are conspicuous to the naked eye, and have therefore been long known, — as in the eggs of many batrachians, where one pole is pigmented, the other less or not at all. The relation of the poles to subsequent stages of development has also, in some cases, been long recognized. Hence have arisen for them the numerous designations *animal* and *vegetative, active* and *passive*, *formative* and *nutritive.*

There are objections to the employment of most of the names that have been used. Thus, while *active* is an eminently fitting expression for the pole where phenomena in such variety are exhibited, the opposite pole cannot with as much propriety be said to be *passive*, since it is in many cases the seat of peculiar, although thus far unexplained activities. *Formative* and *nutritive* are doubtless preferable to the older terms *animal* and *vegetative ;* but to speak of that pole which embraces the peripheral aster of the archiamphiaster as *formative* is to attribute to its substance a function which is confessedly inapplicable to a considerable portion of it, viz. to the substance of the polar cells, which universally take no part in the *formation* of the embryo. It seems to me, therefore, that the substitution of less contradictory expressions is desirable. I would suggest the use of *primary* and *secondary*,* as being entirely consistent with our present knowledge. The active pole is certainly first in importance, and it probably is the first to be raised from an indifferent condition. The nutritive pole is consequently of secondary importance, as in point of time its phenomena succeed corresponding conditions of the primary pole.†

With the elevation produced by the first maturation spindle, at the very latest, the *form* of the egg evinces its monaxial condition, and the primary pole is prominently specialized. The secondary, on the contrary, usually remains undifferentiated ; a few cases, however, have been observed in which it is characterized by changes of form or structure. Probably the most remarkable is that of Clepsine, so carefully studied by Whitman ; but other instances of a peculiar modification of the secondary pole have been seen, especially in the mollusks, and with in-

* The corresponding radii may also be similarly designated *primary radius* and *secondary radius.*

† Whitman (**'78**[a], p. 20), for example, says of Clepsine : "A short period of unipolar activity is succeeded by a long period of bipolar activity which extends through the cleavage stages. In the latter period the contrast between the two poles is still maintained; for the pole thus far active *still asserts its pre-eminence by taking the lead in actions that repeat themselves later and more sluggishly on the opposite pole. It is as if one pole was trying to mimic the performances of the other.*"

creased attention it is altogether likely much additional information on this subject may be gained.*

Polar Phenomena. — Hatschek (**'77**[a], pp. 524, 525) seems to have been the first to attach a general importance to evidences of polar differentiation in early stages of development. It is probable, he says, that a differentiation exists already in the egg cells of all Metazoa. It is most conspicuous in eggs rich in nutritive material, and less evident in small eggs having little nutritive yolk.

Balfour (**'75**[a], p. 210) had previously called attention to the significance of a polar concentration of the nutritive portion of the egg in the process of *segmentation.* "In none of these (vertebrate) yolk-containing ova is the food material distributed uniformly. It is always concentrated much more at one pole than at the other, and the pole at which it is most concentrated may be conveniently called the lower pole of the egg. In eggs in which the distribution of food material is not uniform, segmentation does not take place with equal rapidity through all parts of the egg, but its rapidity is, roughly speaking, inversely proportional to the quantity of food material."

It is possible that this accumulation of nutritive substance is not only an expression of the polarity so generally observable, but is also, as it were, a mechanical cause of the condition, since the polarity is determined during, and by the method of, the egg's growth. In many cases, at least, — as, for example, in Anodonta, — the acquisition of the food yolk is a one-sided process. It is evident from Flemming's work (**'75**, pp. 93, 94) that in the case referred to the animal pole is the one which is less charged with nutriment, and perhaps this is because it is more remote from the channel (micropyle) along which commissarial activity is maintained.

The influence of the nutritive substance upon segmentation and later stages in development was amply comprehended by Haeckel (**'74**, p. 155, **'74**[a], p. 21) at a still earlier period, and has been utilized in his masterly way to explain the superimposed modifications in the process of gastrulation.† Serviceable as its comprehension has proved in this latter

* Compare Lovén (**'48**[a], Pl. X. Fig. 8), Rabl (**'76**, p. 316, Taf. X. Fig. 4), Hatschek (**'77**[a], pp. 504, 505, Taf. XXVIII. Fig. 1), and O. Hertwig (**'78**[a], pp. 202, 209, Taf. X. Fig. 5, Taf. XI. Fig. 4).

† "Unter den secundären coenogenetischen Erscheinungen aber, welche den primären palingenetischen Entwickelungsgang der Keimformen verdecken und fälschen, sind wieder vor Allen wichtig die Einflussreichen Verhältnisse des *Nahrungsdotters* im Gegensatz zum Bildungsdotter." — Haeckel **'75**, p. 404. See also pp. 416–419.

direction, it is doubtful if the localization of the food material is competent to explain all the polar phenomena of the egg. In early stages these may find expression in a variety of manifestations. The migration of the germinative vesicle toward a definite point in the surface; the radial position assumed by the maturation spindles; the waves of constriction which precede the formation of the polar globules, and the inequality in the sizes of the latter; the union of the pronuclei at a point nearer the primary than the secondary pole and the consequently (?) eccentric position of the first segmentation spindle; the appearance of the first segmentation furrow earlier at the primary than at the opposite pole; the formation of pseudopodia-like elevations, — often most conspicuous at the primary pole; the accumulation of finely granular protoplasm at the *secondary* pole after the elimination of the polar globules; and the appearance of "polar rings" and "ring rays" (Clepsine) at both ends of the primitive axis, — are all indications of a polar differentiation of the egg. The eccentric position of the germinative vesicle might in many cases be induced by a regulated distribution of nutritive substance, and the point at which the polar cells appear might be predetermined by the relation of the egg to its sources of nutrient supply; the direction of the wavelike constrictions, the region of greatest pseudopodal activity, and even the position of the spindle axis, might also be dependent on the same (nutritive) conditions. But it must be in opposition to the obstructive properties of the nutritive substance, that the elevation of protuberances at the secondary pole, the formation of the aboral "polar ring," etc., take place.

A differentiation in the substance of the egg at a period preceding the accumulation of deutoplasm, and regulating its distribution, must probably be assumed, and to this are to be referred the polar phenomena which appear later. What may be the immediate cause of this hypothetical earlier differentiation remains to be discovered. It is possible that *the topographical relation of the egg* (when still in an indifferent state) *to the remaining cells of the maternal tissue from which it is differentiated has an important influence in determining this axial condition.* It would certainly be interesting to know if that phase of polar differentiation which is manifest in the position of the nutritive substance, and of the germinative vesicle, bears a constant relation to the free surface of the epithelium from which the egg takes its origin. If, in cases where the egg is directly developed from epithelial cells, this relationship were demonstrable, it would be fair to infer the existence of corresponding, though obscured relations, in those modified cases where (as,

for example, in mammals) the origin of the ovum is less directly traceable to an epithelial surface. Although there are not many existing observations which are sufficiently connected to allow a definite conclusion to be drawn from them in this particular,* there are evidences which in many cases point strongly to the existence of the relationship suggested.

One of the results of a polar differentiation of the primitive axis is often a difference in the specific gravity of the hemispheres, but it has not been found that the conditions are the same in different animals. Thus the primary pole is lighter in the eggs of frogs and the fowl, and it has recently been shown to be the same in certain fresh-water pulmonates (Rabl '75, p. 223), in Unio (Rabl '76), and in Clepsine (Whitman '78[a], p. 29); while in a Gadoid studied by Haeckel ('75, p. 436) it was found to be heavier than the secondary pole, thus causing the more active hemisphere to be directed downward during the early stages of development. In the study of fish eggs, probably nearly related to those seen by Haeckel, Ed. van Beneden ('78, p. 43) observed a slightly modified exemplification of the same thing.

The activities of the primary pole are partly of a more constant nature and widely disseminated, partly either less regular and unlike in different animals, or perhaps entirely absent. To the former belong the production of polar globules, which will be considered later. Of the less constant phenomena, perhaps that which has been most often described is the pseudopodia-like elevation of portions of the cell protoplasm. This, however, may be only a special manifestation of a more general amœboid activity, not always resulting in the formation of pseudopodia, but usually causing the concentration of the active portions of the egg at special epochs in its development. Such concentrations are especially conspicuous in cases where there is an early and sharply expressed separation of the formative from the nutritive constituents of the yolk. This has been observed, for example, in the Ctenophoræ just before segmentation by A. Agassiz, Kowalevsky, and others; in osse-

* A manifest obstacle is encountered in the difficulty of determining after its detachment the position which the egg held during its growth. Unless the germinative vesicle is prominently eccentric both before and after the liberation of the ovum, or the deutoplasm is conspicuously segregated before maturity, or the envelopes of the egg present some differentiation (as, for example, a micropyle) which can serve as a constant point of reference, it may be impossible to reach a satisfactory conclusion. Observations of an axial differentiation in liberated eggs are sufficiently numerous, but the orientation of the differentiated axis in the *ovarian* egg has rarely attracted the attention of observers.

ous fishes before fecundation by Reichert, Oellacher, Van Bambeke (**'76**[a], pp. 1–12), and many others. In the less conspicuous form of a gradual migration, it may be as nearly universal as the occurrence of polar globules. Even in Limax, where no evidences of irregular pseudopodal projections have been seen,* the primary pole becomes clearer after the formation of the maturation spindle, and with the third act of cleavage the antithesis between the two hemispheres reaches a maximum.

Whitman has concluded, from the position of the nuclear structure near the primary pole, that the pseudopodal phenomena in Hydra, as well as the "Faltenkranz" of amphibian eggs, which he considers due to the same cause, are to be classed with the radial arrangements of the yolk during cleavage, and consequently to be referred to nuclear influence; just as the place where the segmentation furrow is first to appear depends on the location of the amphiaster, being always manifest earlier on the side of the yolk toward which the segmentation aster is most nearly approximated. As regards cleavage, I believe he is right in saying that the same relation is probably true in *all cases*, — at least, I have met with only two or three instances which seem to conflict with that rule.† I also agree with him in believing the phenomena have a causal relation. He thinks the pseudopodia are most pronounced, and the cleavage first expressed, at the primary pole, because of *nuclear* influence. But he does not explain why the nucleus has this eccentric position, — why it is near *this* pole rather than some other point of the periphery. The nucleus appears ultimately to assume a position of equilibrium, not with regard to the whole mass of the egg, but in respect to its active constituents. Is not, then, this peculiarity ultimately, though indirectly,

* P. S. — The later observation reported on p. 180, foot-note, serves to show the inconstancy of the pseudopodal phenomena. I believe they can be considered only as special manifestations, whereas the polar concentration of active substance is of more fundamental significance.

† Kowalevsky (**'75**, Taf. XXXVIII. Fig. 14) represents a stage in the segmentation of Pyrosoma where the furrow seems to advance first from the side opposite that occupied by the nuclear figure.

To judge from the figure given by L. Agassiz (**'62**, Pl. XXXI. Figs. 2, 3[b]), one might expect to find the first cleavage furrow in Laomedea amphora appearing earlier on the side opposite the peduncle, since in Fig. 2 the germinative vesicle lies nearest that side of the egg. The natural though not necessary inference is that this side corresponds to the primary pole. But it is stated (p. 313) that "the process of segmentation . . . . commences by forming a furrow across the yolk on that side which lies next to the peduncle of the medusa."

See also the account (p. 419) of Giard's statement for Salmacina.

referable to the want of a uniform distribution of deutoplasm, — to the polar concentration of the protoplasm, in other words?

If the pseudopodia are attributable to the same influence as that which produces the stellate figures of the yolk, they may not of necessity be the direct result of nuclear influence. But of this I shall speak more at length when considering the nature of asters. In Hydra, they are manifest during the first and second cleavage, — less prominently afterwards. In osseous fishes, according to Oellacher and Van Bambeke, they occur before fecundation.* Kupffer and Benecke have described for Petromyzon, beside more regular changes in the form of the primary pole, a protrusion of clear protoplasm, which may be comparable with the pseudopodia seen in other animals. According to these observers, it is both connected with the elimination of the second polar globule, and supplements the act of fecundation. Many observations, especially on the Porifera by Haeckel and Schulze, tend to show the active amœboid condition of the growing ovum; but in these cases the differentiation of the primitive axis appears to take place comparatively late, so that a direct comparison with later and more specialized manifestations is not permitted.

The entirely unique phenomena of "polar rings" (see Whitman, **'78**[a]) are in so far worthy to be classed here as they are special accumulations of active protoplasmic (nuclear?) substance, which manifest themselves soon after the formation of the polar globules. The "ring rays," which stretch out from them toward the equator at the surface of the yolk like the pseudopodal filaments of many rhizopods, and the final migration of their substance inward toward the segmentation amphiaster, afford ample evidence of the active nature of their substance. But how the dispersion of this substance in "ring rays" can be due to nuclear attraction is not clear.

The changes which have been observed at the secondary pole are not numerous. The most interesting is that of the "aboral ring" and its rays in Clepsine. The others are limited, so far as I know, to the elevation of masses of active protoplasm, and their appearance is as unaccountable as that of the aboral polar ring.

By the figures which O. Hertwig has given of this phenomenon in Mytilus, one is reminded of the description which Bobretzky (**'76**, p. 102) has given of the early stages of segmentation in Nassa; and it may perhaps be of significance in the study of these polar phenomena, that there

* P. S. — As has been stated (p. 180), they may occur in species of Limax *during the formation of the polar globules.*

is in this case a complete separation and subsequently a confluence of the vegetative blastomere with one of the smaller cells at the animal pole.

The equatorial zone of clear protoplasm occasionally seen in the eggs of Limax (see p. 183) is possibly another phase of the protoplasmic activity which is usually manifest about the poles of the primitive axis. But all these phenomena have been too little observed to afford grounds for deciding on the nature of the forces which produce them.

ASTERS. — Stellate figures make their appearance in connection with two processes, — cell division, and the formation of the male pronucleus.*

* The aster which accompanies the origin of the female pronucleus is one of the asters of a cell division. It is possible that this aster is entitled to a separate designation, — female aster. The accounts given by Fol (**'77**[c], pp. 450, 451) and O. Hertwig (**'78**, p. 166) for the starfish are not in complete agreement. Fol says of the aster which remains in the yolk after the elimination of the second polar globule, "Il ne tarde guère à s'effacer et à se changer en une ou deux petites taches," etc., and subsequently he says, "Les stries radiaires, peu accentuées du reste, que l'on remarque autour du pronucléus en voie de croissance s'effacent et l'ovule entre maintenant dans une nouvelle période d'inactivité." Although he does not say definitely that there is no connection between these two stars, I think it is fair to assume that to be his belief, for he gives a figure of an intermediate stage (Fig. 12) in which no rays are represented. Hertwig, on the contrary, does not figure any stage in the early growth of the female pronucleus which is destitute of rays. In the text he says that a quarter of an hour after the formation of the second polar globule the homogeneous place existing in the egg, [the "area" of] the internal half of the amphiaster, has increased in size and retired somewhat from the surface. A number of small vacuoles arise in this homogeneous substance. In its vicinity the protoplasm toward the centre of the egg has assumed a radial condition. The vacuoles increase in size, the radiation in their vicinity becomes more distinct, and stretches out farther into neighboring parts. During the confluence of the vacuoles, and the migration of the resulting Eikern toward the centre of the yolk, the rays become less distinct and finally disappear. I find in his account no ground for supposing that the rays about the female pronucleus have an origin distinct from the deep aster. The nucleus, it is true, is represented by both authors as occupying the *centre* of the radiation. That certainly is not the relation in ordinary cell division, and in so far a distinction is justified. The fact that this radiation as described by Hertwig increases during the migration of the pronucleus and then diminishes, also seems to warrant one in ascribing to it a significance different from that prevailing in ordinary cell division. In Limax I find nothing to support Fol's view of the separate origin of a female aster. The internal aster of the second archiamphiaster increases in extent toward the end of the formation of the second polar cell, and possibly after its detachment also; but whether this warrants a fundamental distinction from the asters of ordinary cell division seems very doubtful. There is little in the conditions shown by Limax to support any argument drawn from the concentric position of nucleus and aster (compare Figs. 57 – 60, 68, 72). Most of the investigations on other animals afford even less evidence than the starfish of the separate nature

These two asters are, however, in so many points alike, that one is warranted in considering them, for the present at least, as the results of like processes. The relation which the centre of the aster sustains to the growing nucleus in cell division cannot be urged as the basis of even a topographical difference, for Fol has recently reported a condition in the case of the *male* pronucleus of Sagitta, which shows conclusively that the centre of the male aster does not *necessarily* coincide with the centre of its nuclear structure, any more than new nuclei are coincident with astral centres in division.

The most detailed, and, so far as one can judge without personal observation of the same object, the most accurate description of the changes introducing the nuclear metamorphosis is that given by O. Hertwig for the germinative vesicle of Asteracanthion. The first changes are observed in the *protoplasm* which surrounds the vesicle. The protuberance of protoplasm which invades the vesicle has a clear spot near its apex free from granules, and it sends out long protoplasmic ridges which encroach upon the vesicle. Although he intimates that the first small aster

of the female aster. There is, however, another instance, Sagitta, in which the female pronucleus is represented as occupying the centre of an extensive radial system (O. Hertwig, '**78**[a], Taf. X. Fig. 11). Fol, however, makes no mention of such a system, which seems the more remarkable as he observed the peculiar condition of the male aster in Sagitta.

Another radial figure, that which surrounds uniformly the conjugated pronuclei, may also possibly be a separate phase of the astral phenomenon. For the present, however, I believe it may safely be regarded as a continuation, and perhaps an extension of the so-called male aster. O. Hertwig ('**75**) has described it in Toxopneustes (pp. 400, 401) as though it might be genetically connected with the two asters which arise at the first segmentation, as well as with the male aster, but in his general conclusions (p. 416) he has very definitely stated that this old single nucleus is *dissolved*, and that the asters of segmentation arise as *new* structures. Hertwig and Selenka agree in making the male pronucleus much smaller than the female when they come in contact, and Selenka has recently come to the conclusion that the former continues to increase in size until it equals the latter before there is a real fusion of their substances. May it not be that the extensive radial system surrounding welded but unfused pronuclei is only a male aster which ceases to exist when its nucleus has attained normal dimensions? It is possible that the aster of the female pronucleus, when such exists, shares in the production of this central sun. The entire absence of both male and female asters in the case of Limax might perhaps in that event be a sufficient explanation of the non-appearance of a conjugation aster; but it cannot be denied that the fusion of male and female pronuclei might also generate a force capable of inducing similar radiations, for there is reason to believe that their substances are sufficiently unlike to exert a mutual attraction.

But if either of these asters is constantly developed, it remains yet to discover the means of making their rays visible.

arises *subsequently* in this protuberance, I cannot think these radial ridges are due to anything different from that which causes the true asters. The appearance of one or both the asters seems, then, to be the first change in the approaching metamorphosis. That is entirely consonant with the observations made on the nuclear metamorphosis preceding the first cleavage in Limax. It is certainly a matter of no great importance whether the invasion takes place a little earlier or a little later in the history of the formation of the asters. In Limax the asters are often so far removed from the nucleus that they must attain considerable size before any conspicuous changes are effected in the latter. In other cases, as, for example, in the pteropods as shown by Fol, the earliest evidence of the existence of a star is to be seen within the outline of the nucleus. I do not conceive, however, that cases like the last really conflict with the conclusions just stated. The transparency of the nucleus may be in itself enough to explain the detection of rays through its substance sooner than in the surrounding protoplasm. Fol's (**'77**[c]) account of the metamorphosis of the germinative vesicle in Asterias glacialis seems to indicate that the asters arise at a much later period, namely, after radical changes have taken place in the germinative vesicle and in the germinative dot. It is without doubt one of the most delicate and difficult of the questions connected with maturation, to ascertain when and where the first traces of the archiamphiaster appear. But unless this author's final paper brings strong evidence to show the inaccuracy of Hertwig's observations, it seems to me we may accept the latter as entirely trustworthy in this particular. Certainly the figures accompanying Fol's preliminary paper in no way invalidate the evidence given by Hertwig, for in the earliest stage figured in which acids had been employed (Fig. 5) not only are *both* asters formed, but the spindle is represented and also its equatorial thickenings. But that condition represents a stage much advanced beyond the first appearance of the first aster. The want of evidence that asters exist in the stages represented by the figures which precede may be due to their all having been made from living eggs. It can scarcely be doubted, for example, that the stage shown in his Fig. 4 is more advanced than that exhibited in Fig. 5, since the oblique or tangential direction of the spindle (Fig. 5) precedes rather than follows the radial position (Fig. 4). Concerning the statement that the amphiaster is formed *within* the germinative vesicle, or what remains of it, but is from the beginning eccentric in position, I can only say that the drawing (Fig. 5) is not sufficient to prove that the *centres* of the asters lie within the finely granular territory which I take to be the remains of the ger-

minative vesicle; and even if it did show this, it would not follow that such a position could not have been effected by the invasion of an aster-bearing protuberance of vitelline protoplasm, in the manner described by Hertwig.

There are no accounts by other authors in which the centre of the aster is shown to lie within the nucleus, — none in which it is not possible to suppose that the protoplasm surrounding the nucleus takes at least an equal share in the formation of the asters. From the cases given above it will be sufficiently clear that the converse of this proposition does not hold true. So far, then, as regards the origin of asters, I hold that they are primarily phenomena of the protoplasm rather than of the nucleus. I do not wish, however, to deny to the nucleus the possibility of any influence in their production, but must insist that the immediate cause of their appearance is *not of necessity a morphologically persistent part of the nucleus.*

The male aster appears to present the most serious obstacle to this view of the origin of molecular stars. If it be granted that they are essentially like other asters, it may pertinently be asked what evidence there is that the star is not due to the direct influence of the nuclear substance (male pronucleus), toward the centre of which its rays are directed. From my own observations on Limax I should hardly be able to give any satisfactory reply; the only asters which I should feel justified in referring to the influence of the male element are those which occur in the single abnormal case described. The short rays are there directed toward central corpuscles, which I have assumed to be equivalent to the male pronuclei seen by other observers, so that the evidence, little as it is, would be unfavorable to the view I have adopted. But Fol's ('**77**[c], p. 465) observation above alluded to may possibly offer an explanation of the difficulty, and ultimately prove that the male aster is, after all, only an apparent exception. Fol states that in Sagitta, during the motion of the male pronucleus toward the female, it is very evident that *the centre of the star* (male aster) *is in advance of the clear spot* (male pronucleus), *and that the latter is drawn on in a passive manner.* The figures which O. Hertwig has given of the pronuclei and their asters in Sagitta do not, it is true, *directly* confirm this observation, but the pear-shaped outline of the pronuclei, when compared with similar forms which are shown in Limax (Fig. 68) to be probably due to the attraction of an aster, is sufficient to suggest the possibility that this peculiar contour in Sagitta has been produced by a like cause. Unfortunately, Fol has not stated whether the pronucleus suffers any change of form as

a result of the traction; but taking into account Hertwig's figures and what I have seen in Limax, I am inclined to think that all are due to the same cause, and that the male aster centres, as Fol claims, in advance of the nucleus, and at least may induce its elongation. In that event, the exact centre of radiation has not been clearly seen by Hertwig. If so experienced an observer has overlooked the true relation of nucleus and aster in so favorable a case as that of Sagitta, it will not be too much to say that renewed observations directed especially to this point may prove that the relation of aster to nucleus has hitherto been only partially comprehended.

Whether, however, the formation of asters can really be regarded as the first visible alteration in the nuclear metamorphosis, may still be open to question. There are many descriptions of important changes occurring in the nucleus prior to the detection of any stellate figure. Especially in the metamorphosis of the germinative vesicle is this the case. But, from one cause or another, most of these descriptions cannot be considered as definitely excluding the possibility that stellar figures accompany or precede the indicated changes. The very careful account of the metamorphosis of the germinative vesicle in Asteracanthion given by Van Beneden, for example, affords no means of deciding this question, since he failed to discover the asters at any stage. The necessity in most cases of using reagents to demonstrate the stellar rays, makes all continuous observations on living specimens of little or no value in endeavoring to ascertain the synchronism of the astral phenomena and the changes within the nucleus. Were the first detection of rays in *living* eggs equivalent to finding the very beginning of such structures, the question would have been long ago definitely answered by Auerbach's studies, for he recorded the disappearance of the nucleoli at the time of the confluence of the two pronuclei, and observed the stellate figures only at a later period. But the timely use of reagents would probably have shown the existence of asters at stages as early as those in which they are found in Limax.

A possible objection to the view that stars introduce the nuclear metamorphosis is presented in the division of tissue cells, where astral figures of the protoplasm are less pronounced or altogether invisible. Here the relatively great size of the nucleus, and the prominence of its labyrinthine filaments are such as to make a study of the radial appearances in the cell protoplasm much more difficult and unsatisfactory than in early embryonic cells. But even here, according to Flemming's studies on the epithelium of the salamander, centres of attraction are found at the poles

of the nucleus during the first phase of the intranuclear changes.* Although the plane of division is seldom inclined sufficiently to allow one to look, even obliquely, on the pole of the prospective spindle, yet stellate arrangements of the scanty pigment granules and fat globules of the protoplasm are to be seen. These Flemming compares, with justice, to molecular asters. It follows from his observations, I think, that we have as yet no grounds for presuming that the stellate figures in tissue cells are dependent on preceding alterations of the nucleus, — certainly not that they are brought about by a segregation or localization of nuclear substance. There is nothing in Flemming's figure of this stage (Taf. XVI. Fig. 2 *a*), nor in the text, to indicate that there is at this time any evidence of a dicentric arrangement on the part of the nuclear filaments themselves. In cases where, from the absence of granules in the protoplasm, these asters are not rendered visible, it is none the less probable, as Flemming maintains, that they exist.

But there are cases in which the division of the nucleus is in all probability not accompanied by such fundamental rearrangements of its substance as appear in the various modifications of the spindle figure. Thus far, I believe, no trace has been found of molecular asters in these instances of direct nuclear division. That, however, does not warrant the conclusion that asters, being formed about a segregated portion of the nucleus, are here wanting because no such localization of nuclear substance has taken place. The only inference which seems to me justifiable from this evidence is, that the filamentous and other differentiations of the nucleus are correlated with the existence of molecular asters. It affords no means of ascertaining the nature of this relationship, and therefore is without significance in any attempt to answer that question.

As regards the evidence to be drawn from the so-called free nuclei, it is too limited to be of great value. The view that these nuclei do not arise *de novo*, but result from the division of previously existing nuclei, has only recently been gaining support, and there are not many observations on their division. Whitman (**'78**[a], p. 272) has "seen these nuclei (his entoplasts) pass through the successive forms of a dividing amphiaster," and Balfour (**'78**, p. 17) has shown that his "yolk nuclei" sometimes present much the same appearance of a double cone as do the nuclei in the germinal disk. While the former observer certainly saw the astral figures, the latter, it must be concluded, did not, for he gives

* "Wichtiger ist eine *innere* Veränderung im Zellenleibe: Schon in diesem Stadium (1. Phase) existirt in ihm, wie ich kurz sagen will, *eine dicentrische Anordnung*, den künftigen Theilungspolen der Kernfigur entsprechend." — Flemming, **'78**, p. 372.

no representation of them in his figures, and says in the text (p. 24) that these conelike nuclei of the yolk exert no influence on the surrounding protoplasm. It is perhaps impossible to draw a conclusion of universal applicability from these accounts, but it will be granted that it is possible for stellate figures to accompany the division of nuclei in syncytia, as well as in definitely limited cells. The existence of asters in syncytia once established, it still remains to be ascertained whether they will cast any light on the supposed share which the nucleus takes in their production, or on the nature of the influence they exert upon the nucleus during its division. For the present I see no reason to anticipate the necessity of modifying the views I have arrived at from a study of *cell* nuclei.

Plant cells rarely afford the opportunity of studying the radial phenomena in the protoplasm during nuclear division, as Strasburger and others have already pointed out. Why the centres of attraction exert apparently so little influence on the protoplasm, it is difficult to say. The great size of the nucleus as compared with the mass of the cell protoplasm, and the vacuolation of the latter, are features which restrict the possibility of well-marked asters. Certain it is that they are not so clearly defined as in animal cells, though there are evidences of a radial tendency in the protoplasmic filaments of Spirogyra, etc. The poles of the spindle are usually so near the surface of the protoplasm that there is little opportunity to form extensive rays. Cases where the centres of attraction lie wholly outside the nuclear structure in plants (Isoëtes) are not numerous, but I cannot think such unfavorable objects as plant cells and animal tissue-cells are competent to cast doubt on the nature of what is so evident in the segmentation of eggs. Strasburger gives assurance that in division the nucleus first becomes homogeneous, and then a contrast is developed between two opposing points of its surface. The latter are doubtless equivalent to the astral centres, and the difference in the order of events as compared with Limax may possibly be explained as resulting from the inconspicuous nature of the asters in plant cells, whereby their earliest stages have been overlooked.

In what precedes I have endeavored to show the possibility of a much earlier origin for the asters than has generally been recognized, — that they precede the disintegration of the nucleus, and are therefore to be looked upon, not as the result of a segregation already effected in the nuclear substance, but as the seat of forces actively engaged in remodelling the constituents of this central body. So long as it remained undisputed that the centres of the asters lay within the nucleus, or at

its boundary, no valid objection could be raised to considering them (the centres) as localized portions of the nuclear substance, — the less objection since their deportment under the action of staining fluids was such as is exhibited by nuclear substance. Now the case appears somewhat changed. The fact that they may lie at some distance from the nucleus while the membrane of the latter is still intact, seems to preclude the possibility of any formal elements of the nucleus taking part in their initiation. It does not, however, prevent the supposition that fluid portions of the nucleus may have traversed its membrane, and have been recondensed, so to speak, in the form of "areal corpuscles." Still these corpuscles, when they exist, do not stain as deeply as the nuclear disks, and are possibly only condensed portions of protoplasm. The nature of their staining, however, indicates that they probably are composed exclusively of neither nuclear substance nor cell protoplasm, but are produced by a fusion of the latter with fluid constituents of the nucleus. The position of the centres of attraction constantly in the vicinity of the nucleus, rather than in remote parts of the cell, is indirect evidence that the nucleus exercises some influence in their production. But one is incapable of saying why the asters appear at particular points, and why there are just two of them. That they do not appear at the same instant is evidence that they are to a certain extent independent of each other. The regularity with which they arise in positions definitely related to the main axis of the egg at the first division, and to the plane of the last preceding division in subsequent stages, shows clearly that their location is controlled in accordance with fixed laws, and it may be reasonably conjectured that the distribution of the active protoplasm (or, what amounts to the same thing, the position of the nutritive portion of the yolk) is an important factor in determining the law. Yet it is not the only factor; since in the first division of Limax, for instance, it might determine in what one of an infinite number of latitudinal planes the asters should lie, but it probably could not influence the selection of any one of the infinite number of diameters in that plane for the astral pair. The latter might possibly be effected by the direction from which the male aster approaches the female, and thus its determination be ultimately referred to an entirely fortuitous circumstance, — the location of the point where the spermatozoön effects an entrance into the yolk. This, however, I doubt, since I have not been able to conclude that the asters have any fixed position in relation to the two pronuclei or their plane of contact. If, as Fig. 79 seems to show, one of the asters could make its appearance before the contact of the pronuclei, it is difficult to

conceive what influence the mutual relation of the pronuclei could have in determining the place of the two stars. From my observations on Limax I am of the opinion that the pronuclei exercise only a limited influence on the position of the first amphiaster. As the centres of its stars may lie deeper in the yolk* (farther from the animal pole) than the pronuclei, I am induced to think that their positions are determined by some unknown influence which probably resides in the protoplasm itself, and in this I see another reason for hesitating to consider the asters as the result of an attraction exerted by a part of the nuclear body on surrounding protoplasm.

The theory that the stellate figures are due to the outstreaming of nuclear fluid from the nucleus undergoing division or disintegration, leaves the existence of *male* asters without an explanation, for there is no pre-existing accumulation of nuclear fluid to be thus put in motion. Even as applied to the nucleus undergoing division, it is confronted by serious obstacles, some of which I have already (p. 286) stated. As with every other theory which involves a circulation of fluid, the results appear disproportionate to the protracted period during which the supposed "flow" is maintained. There is no accumulation of the clear (nuclear?) fluid at the peripheral ends of the rays such as might be expected to result from so long continued a current, and when the rays ultimately disappear it is first at their peripheral ends, — not at their central ends, as would naturally result if there were an outflowing stream. Apparently the only way of explaining this method of disappearance, in keeping with the theory of centrifugal currents, is by assuming that the nuclear fluid in the rays becomes diffused through the yolk, and that this diffusion begins, or proceeds more rapidly, at the distal ends of the rays, thus inducing their earlier obliteration near the periphery. But, if that were possible, might not the original distribution, as readily as this, have taken place as a uniform diffusion through the whole yolk without engendering any astral figure? Certainly the rapidity with which the stars grow is not so much greater than that of their disappearance as to make it possible for simple diffusion to accomplish one, and not the other. It is also incredible that fluid forced from the tips of an elongate nucleus at so slow a rate as must be conceded, should exhibit such fineness and such marvellous uniformity in the nature and the direction of its currents, unless some pre-existing structural condition determined its course.

But the principal objections to this view, which was first advocated by

* See Figs. 85-89.

Auerbach, are the facts that *the asters have been shown to arise at a distance from the nucleus, and before the latter had suffered recognizable diminution in volume.*

The idea that the asters are the optical expression of currents of clear protoplasm setting toward the "centres," has much more in its favor than the theory of centrifugal currents. It does not conflict with the view, in support of which there is much evidence, that these are centres of attraction, and it is readily harmonized with the fact that there is an accumulation of clear substance — the "areas" — about these centres, which seems to present the same properties as the substance of the rays. It, nevertheless, appears insufficient to explain all the observed phenomena. If the star is due solely to centripetal currents, it is unintelligible how it should attain such a size as it often does without a corresponding accumulation of clear substance at the centre, or why, on the other hand, "les rayons de l'aster mâle ne commencement à se montrer nettement que plusieurs minutes après la fécondation et lorsque la tache claire s'est déjà avancée un peu vers l'intérieur du vitellus." (Fol.) Another obstacle to this view is the deportment of the rays in certain special conditions of the aster. If the spiral course which they sometimes exhibit is induced by any mechanical or other influence *after* their formation,* it seems impossible to explain them as simply currents of either protoplasm or nuclear fluid, since any extraneous force which could produce such extensive alterations in the direction of the rays would obliterate so susceptible a thing as a stream of fluid. But whatever may be the conclusion respecting the rays of the spiral asters, the lateral deflection of those constituting the peripheral star during the formation of the polar globules is evidently the result of a mechanical influence. They are secondarily altered in direction, but maintain their individuality, notwithstanding the pressure which prevents their assuming a rectilinear position. If in this case the rays were only streams of fluid, the resistance offered by the egg envelope would merely result in shortening them without causing any such modification of direction as has been repeatedly observed. The astral rays are visible in virtue of their possessing different refractive power from the intervening portions of the protoplasm, not simply by reason of the

* If it were assumed, on the other hand, that the spiral condition was *not* superinduced, but was from the beginning of their formation characteristic of all asters in which it is found, it would be difficult to explain why the currents followed such a systematic and yet indirect course, unless one assumed in addition the pre-existence of a special structural condition of the protoplasm determining the direction of the currents. But there is nothing else to favor this latter assumption.

displacement of yolk granules. The assumed motion is, therefore, not enough to account for the appearances; it offers no explanation of this difference of refraction. No satisfactory explanation of the cause of the latter will necessarily exclude the possibility of a motion, but it cannot rest on that assumption alone; for however definite the course of the flow, it could produce no such optical effect until there was a differentiation into more refractive and less refractive portions.

If the aster is only the optical expression of currents in the protoplasm, it remains to be explained why it is that such currents do not uniformly produce this effect. There certainly may be a flow of substance without astral figures. The male pronucleus in Limax, for example, grows within a short time to a comparatively large size without necessitating the existence of any astral phenomena, and yet it cannot be doubted that it grows at the expense of the substances of the yolk. There is no reason to suppose that it has greater power than the male pronuclei of other animals in rendering assimilable the substance in its immediate vicinity, and that it may therefore dispense with far-reaching protoplasmic currents which are necessary for *their* growth. If there are currents in the one case, there doubtless are in the other, and their magnitude and velocity, if proportionate to the rapidity of nuclear growth, will not be less in Limax than in the average of other cases. On the other hand, asters may possibly remain for a time unaccompanied by corresponding movements of clear protoplasm; at least, there are often great differences in the size of the areas which form the centres of asters having nearly the same extent.*

It is not to be overlooked that the areas may not be exclusively due to an accumulation of clear protoplasm, and thus to an indirect repulsion of the granules of the yolk. It has not yet been shown that it is impossible for the granules to have been employed in the chemical changes presumably taking place at the centre of the aster, — that is, that their disappearance from the "area" may not be due as much to an actual chemical alteration as to a mechanical dislodgment.

The subsequent occupancy of the region of the central area by granular protoplasm is an argument neither for nor against the physical displacement of granules from the area. The clear substance is at least largely consumed in the growth of the nucleus. As the latter does not migrate far enough to have its centre occupy the place of that of the aster, this consumption of areal substance necessarily implies its re-

* Compare Limax, Figs. 73 and 80[a], with Fig. 85; also O. Hertwig, '75, Taf. XIII. Figs. 21 and 23.

moval from the area, and this motion is compensated by a corresponding (centripetal) movement of the granular protoplasm. The same, it is true, could not be claimed for the clear *rays*, for their place is subsequently occupied by granules which it is fair to presume were simply displaced during the astral manifestation, since the granulation of the yolk is not permanently diminished by their formation.

I do not claim that there is absolutely no transfer of substance to and from the centres of attraction,— on the contrary, I believe the phenomena are, on any other assumption, unintelligible; but it seems to me that the formation of a clear area and the existence of radial striations are far from commensurate, and that to claim that the rays are only the optical expression of currents is to associate as cause and effect two things which have not necessarily any such connection with each other.

The view suggested by Strasburger, that the rays are evidence of the *polarity* of the protoplasmic molecules, seems to imply that the astral condition is effected by the molecules having the direction of their principal axes so altered as to be radial, — that is, practically parallel to each other, — this position being maintained by the (attractive?) influence emanating from the so-called centres. But the *spiral* asters (unless they are superimposed conditions) appear unexplainable upon this hypothesis, since the direction of the force must always be strictly radial, and the attracted molecules could assume all intermediate attitudes between the radial and nearly tangential only by the intervention of other forces, of the existence of which we have no other evidence. If the rays are due to the polarity of the molecules, their position must be very unstable, and it will exist only so long as the attractive force continues to be exerted. In hardened specimens the force is of course interrupted, and the astral conditions are preserved only in virtue of being fixed by the reagent before the latter had interrupted the processes generating the supposed force. The *intensity* of the attraction (or repulsion) must of necessity diminish with the distance; but does that warrant the lapse of such an interval as occurs between the time when the central molecules respond to the force and that when those near the periphery give evidence of a like condition? But, further, I do not understand how such an orientation of *all* the molecules could accomplish a difference in the refractive properties of neighboring rays of the protoplasm. It would not be claimed that the molecules are directly visible, or that their alignment was capable of direct observation. The protoplasm would still remain homogeneous.

Bütschli's opinion, that the asters are the optical expression of a

physico-chemical alteration of the protoplasm emanating from the central area, is probably incontrovertible; at least there is a physical alteration of the protoplasm, and it first becomes apparent at the centre of the aster; but this is rather a description than an explanation of the appearances.

I have already dwelt upon some features of the astral phenomena which seem to strengthen the position maintained by Flemming, — that the asters represent a *structural* condition of the protoplasm; but that simply implies a greater stability in the nature of the rays — a closer approximation to a solid condition — than is generally maintained, and offers not the least explanation of the cause.

The substance which composes the central portion of the asters accompanying division was uniformly described by the earlier observers as an accumulation of *homogeneous* protoplasm, and as such it always appears in the *living* egg. Flemming was the first to show that a portion of this "central area" is differentiated as a corpuscle capable of a slight degree of staining, and for that reason he took the corpuscle to be the beginning of a new nucleus. O. Hertwig has also recognized in all his studies the existence of a stainable corpuscle occupying the centre of the area, and has reproduced it in nearly all his more recent figures with almost diagrammatic uniformity as a very minute body in which the spindle fibres terminate. Strasburger has represented nearly the same condition in his revised studies of segmentation in animals. In the opinion of both these observers, the centre of the aster is occupied by a visible portion of the old nucleus. The evidence that this corpuscle is nuclear substance they find in the constancy with which it is stained, as well as the fact that it forms the tip of the old nucleus when the latter is drawn out into the form of a spindle.

My own studies lead me to believe that different reagents are not uniform in their effects, and I would refer to this some of the various conditions in which the corpuscle has been exhibited by the preparations in the case of Limax. It is quite improbable that all the variations are thus referable, but it cannot be doubted, I think, that certain acids are much more likely than others to make visible a differentiated central corpuscle. In acetic-acid preparations the whole area has generally appeared nearly homogeneous and usually not well defined, but sometimes (Figs. 22, 25) quite sharply limited and free from every trace of internal structure; in a few instances, as though composed of a flocculent mass (Fig. 55). In certain stages of the archiamphiaster (Figs. 43, 48, 50) it has been occupied by a large more or less flattened corpuscle, which

was nearly as extensive as the whole "area"; and, finally, at an early stage in the formation of the first segmentation amphiaster, by a few scattered (Fig. 85), or more definitely grouped (Fig. 82), refringent corpuscles. Since these various conditions exist in eggs that were subjected to nearly the same treatment, it is not possible to account for the differences as due to the action of the acid. Still, the nearly homogeneous condition is the one by far the most prevalent with the employment of acetic acid. With osmic acid, which according to Hertwig is the most satisfactory to demonstrate the existence of nuclear substance, I have not uniformly succeeded in showing a central body, but with chromic acid a small, lustrous, sharply limited corpuscle is almost always distinguishable (Figs. 44, 52) exactly in the centre of the radiation. A comparison of Fig. 52 with Figs. 73, 79, and 80 will illustrate the differences which result from treatment of the same stages with different reagents. It may possibly appear significant of the accuracy of Hertwig's view of the nature of this areal corpuscle, that it is already differentiated at the earliest stages in the formation of the aster which I have seen. I cannot deny, in those cases where the nucleus elongates and its poles are observed to occupy the centre of the aster, that the most natural inference is that the corpuscles are segregated portions of the nuclear substance, but in the case of Limax the assumption seems impossible. These areal corpuscles lie outside the sharply marked territory of the nucleus, often at a considerable distance, and there is no evidence of a direct connection between the two. From theoretical considerations it may be difficult to explain the activities of the astral centres without admitting a fusion of nuclear and vitelline substances, since it is not plausible that a chemical process should be initiated in a homogeneous substance without the presence of a second material differing in composition from the first; but the mingling of vitelline and nuclear matter does not necessitate the appearance of the latter in the form of discrete corpuscles; besides, the detachment of portions of nuclear substance seems in Limax irreconcilable with the early relations of nucleus and aster. It seems unsatisfactory to consider the areal corpuscle as unmodified "nuclear substance," exercising an attractive influence on the surrounding protoplasm, since the remaining and major portion of that substance gives no evidence of exercising a like influence on the yolk. I am therefore inclined to regard the corpuscles as a product of the fusion of nuclear and vitelline substances.

Their ultimate fate is as uncertain as their origin. There is reason for believing, from evidence given elsewhere, that in Limax the corpuscle

of the external half of the archiamphiaster becomes fused with the envelope of the polar globule at its distal pole. Whether the corresponding corpuscles of the deep aster are also excluded from participating in the formation of the new nucleus is more doubtful, but certain appearances (Figs. 58, 60) favor the view that they may persist as discrete bodies for a long time, perhaps till the new nucleus has acquired a membrane. In that event they could hardly be employed in the nuclear reconstruction without losing their morphological identity. As the area more often appears homogeneous during the later stages of nuclear growth (Figs. 59, 68, 93, 91) it is reasonable to assume that they are ultimately redissolved.

There is one point in connection with the genesis of the asters accompanying the first segmentation which deserves particular attention. If it could be shown that the rays of the "conjugation aster" are gradually altered in direction so that a portion became centred about one of the poles of the first segmentation spindle, and the remainder about the opposite pole, it would be strong evidence in favor of Hertwig's view that the radial phenomena are due to the attractive force exerted by the nucleus upon the protoplasm, and that these forces, at first operating uniformly in all directions, distribute themselves with the elongation of the nucleus to its two poles. But the proof is not yet convincing; on the contrary, it appears as though the asters at segmentation arise quite independently of the "conjugation radiation." The evidence that these asters are due to the direct attraction of nuclear substance appears materially weakened by this want of continuity in their manifestations. While I concur with Hertwig in the belief that there is an attractive force exerted upon the vitelline protoplasm, which emanates from the centre of the radiation, I would suggest that the force is generated by the fusion of two unlike substances, — one of which is vitelline protoplasm, the other probably fluid constituents of the nucleus, — and not by the attractive properties of either. Thus the attraction on the one hand of protoplasm from the vitellus, and on the other of nuclear matter from the nucleus (migration of lateral zones), may be effected by the same force. But if it is *nuclear substance alone* which exerts the attractive influence, how shall it be explained that it attracts the nuclear disks?

Spiral Asters. — I have designated as *spiral* asters certain peculiar conditions often affecting the stellate figures connected with the elimination of polar globules. I have not met with anything in the observations of others which can be classed with these appearances. The rays

of the asters in cell division have often been represented, since the time of Auerbach ('**74**, Taf. IV. Fig. 11), as being at certain stages *curved*, but in none of them is the slight curvature of such a nature as to prevent each ray from lying wholly in a plane. The rays of the spiral asters are often much more prominently curved, and are not limited to a single plane. But they also are not constant phenomena of any given stage, nor of all asters. Therefore, whatever may be the cause of this peculiar arrangement, it is not likely to be of fundamental importance. The spiral condition will probably be instructive only in so far as it throws light on the nature of asters in general, — on the physical state of their rays.

The spiral course of the rays in the *superficial* (polar-globule) aster might fairly be accounted for by assuming that it is caused by the force which urges the tip of the maturation spindle into contact with the envelope of the egg. The spiral form of the external aster would, then, be only *one* of the results of its being compelled to adjust itself to an altered position. What in one case is effected by a simple outward and backward deflection of the rays producing the funnel-shaped figure, may in this case be accomplished or aided by a *lateral* deflection. The spiral course would evidently allow the centre of an aster with rays of fixed length to approach nearer the surface than could otherwise be. If this is the correct explanation, the spiral, like the funnel, results from the force which impels the archiamphiaster against the resisting envelope of the yolk. In one case the adjustment is accomplished by a simple bending of the rays, each of which continues to lie wholly in a plane coinciding with the axis of the spindle; in the other case the same end is attained by the addition of another curvature which takes the ray out of that plane.

This explanation would, I think, be entirely satisfactory if the phenomena were limited to the superficial aster, but it is difficult to conceive how a force (contraction ?), acting in any part of the yolk, could induce such extensive spirals as are seen in the rays of the deep aster of Fig 78. If the rays are the result of a constructive process, one might assume that this construction advances in straight lines till it reaches the periphery of the yolk, and that a deflection is then necessitated on account of the resistance offered by the yolk envelope, — a resistance that is sometimes overcome by the joint action of neighboring rays, which thus cause pseudopodal elevations of the surface. This resistance would then, it may be assumed, be propagated along the existing portions of the ray, — such a transmission being rendered possible by

its semi-solid condition, — and effect an even curvature throughout its length. Such an explanation would not be inconsistent with the S-shaped curves sometimes (Limax, Fig. 57) seen. But the rays show a spiral tendency before they reach the periphery, and those on the side of the aster nearest the surface are no more curved than those on the opposite side (Fig. 66). These are obstacles which are not readily explainable, for it is unsatisfactory to assume that the rays extend farther than they are visible.

But whatever the view adopted regarding the cause of the spiral arrangement of the rays, I believe there is great reason — both from the spiral form and from the more simple deflection of the rays of the external aster — for regarding them as something more than protoplasm in a state of flux. It cannot be positively shown that either of the conditions is not produced by the contracting influence of the hardening reagent until such arrangements shall have been observed in *living* eggs. The same objections, however, hold good against considering these asters artificial products, that have been so justly urged to prove that asters in general cannot have been produced by reagents. Yet it still remains possible to claim that the *particular course* of the rays in these cases has been indirectly caused by the influence of the acids; that, for example, the immediate effect of the acid on the polar-globule protuberance would be to diminish its capacity, and thereby compel the rays to assume some other than the simple straight course they preserved in the living state. The principal objections which can at present be urged against this position are, — (1.) that the surface in this (polar-globule) region shows less evidence of having suffered from a contraction than that of any other portion of the egg, for a diminution in the capacity of the protuberance would imply a folding of its envelope, but that is just what does *not* take place; (2.) that there is a *progressive* modification of the direction assumed by the rays, which corresponds with the advancement attained in the formation of the polar-globule protuberance, so that the least deflection corresponds with the least advanced condition of the elevation. Besides, no reasonable diminution of volume could alone account for the extensive spiral of the deeper aster. I am therefore of the opinion that this phenomenon is not caused by the process of hardening, and that consequently it will eventually be found in *living* eggs.*

* P. S. — Prof. C. O. Whitman of the University of Tokio, who had seen my preparations previous to his departure for Japan, writes me (under date of June 18, 1880) concerning one of his students (Mr. Iijima), engaged in studying the early

Nuclear Spindle. — The fibrous cords which collectively form what Bütschli named the "spindle-shaped body" are intimately connected in their origin with the asters. But to claim that they have only the same significance as the rays of the latter, is not warranted by the observations. They are not only thicker, but they also pursue a different course not strictly radial, and they exhibit special accumulations of readily stainable substance; they are principally composed of nuclear substance, — the rays of vitelline protoplasm. While, then, I cannot agree with Fol that the spindle fibres (bipolar filaments) are not different from the unipolar filaments of the aster, and that they *appear* different simply because enveloped in a different medium, there are still grounds for a comparison. Since the centre of the aster, when it begins to appear, often lies entirely outside the nucleus, the rays must, in such cases, at first be formed exclusively in the yolk, and those which project toward the nucleus are composed of vitelline protoplasm, as well as those which radiate in other directions. The further growth of the aster in the direction of the nucleus is really an encroachment of the vitelline substance on the nuclear territory, just as O. Hertwig has shown to be the case with the germinative vesicle and first maturation spindle in Asteracanthion. Conditions such as are shown in Fig. 85, Limax, also afford strong evidence that *in the beginning the rays which eventually become spindle fibres are formed like the remaining rays of the aster.* They are rays which are formed outside the nucleus, or commence outside, and, as it were, push their way into that structure. But with that invasion is coupled the metamorphosis of the nucleus, so that the latter is not to be regarded as simply a passive participant in the changes. All accounts agree, I believe, in making the formation of the spindle fibres progress from the poles of the spindle. In relation to the centre of the aster, therefore, they grow like other rays, — in a centrifugal direction. Their course, like that of unipolar filaments, is radial, until, by increase of length, the rays from the two stars meet midway to form the continuous bipolar filaments. Their course now becomes slightly bent, so that, collectively, they present the appearance of a cask.

Thus there exist many features suggestive of the identity of astral rays and the initial condition of spindle fibres. It is a fundamental question whether these fibres are ever constituted in any part of vitelline

stages of Nephelis: "His preparations show most distinctly what you discovered in the egg of Limax, — curved radial lines. I can but wonder that Hertwig and Bütschli did not recognize the same."

protoplasm. Their centrifugal growth is not proof of it, but simply makes it probable that the influence exerted from the centre of the aster is *increasing* at the time of their formation, and that the substance of which they are composed is affected in a manner similar to that of the vitelline rays. An objection to this supposition is found in the more common descriptions of the nuclear metamorphosis, in which the asters are located at the boundary of the nucleus and vitellus, the former being elongated into a more or less spindle shape. There is nothing in this to suggest an incursion of vitelline substance; besides, the spindle fibres have been shown, especially by O. Hertwig, to be formed within the nucleus when its lateral walls were still complete. At the ends of the spindle-shaped nucleus, however, in all such cases, the nuclear boundary has ceased to be visible, — the substances of nucleus and vitellus are in contact. It therefore does not appear entirely impossible that an invasion of slender threads of vitelline protoplasm might take place at these points. But if they were just like the rays running through the yolk, one would expect to find them traversing the whole space of the nucleus, and not limited, as they have been shown to be in some cases, to an axial portion.

It appears significant that the aster is never found to lie wholly within the nucleus, but has been found wholly outside that structure. Since, then, in some instances, there is no motive for ascribing to a portion of the rays of the aster, in its early stages, a condition different from the rest, I am led to the conclusion that the rays which stretch through the nucleus are invasions of delicate filaments of protoplasm about which the nuclear substance is progressively accumulated. This may terminate in an intimate fusion of the two substances, or the latter may exist as an investment of the former. I believe that the deportment of the fibres at the time the nuclear disk divides offers some support to the latter view.

The peculiar movements of the nuclear substance are perplexing. No entirely satisfactory explanation of them has been given. With the formation of the spindle fibres there is unquestionably a transfer of this substance, not only to definite tracts indicated by the course of the fibres, but also, and principally, toward the equatorial plane. Do these movements occur in response to a force operating from the centres of the asters? In view of the first appearance of the fibres at their astral ends, this seems a reasonable assumption. The force must, then, be one of *repulsion* for the nuclear substance. But that is not readily reconcilable with the subsequent division of the nuclear disk and the *approach* of

this same substance toward the astral centres. There is nothing in the appearance of the asters to warrant the conclusion that they at first exercise a repulsive, and subsequently an attractive influence on the same substance. Besides, the accumulation of the nuclear substance along other portions of the spindle fibres than the equator seems most naturally explainable as resulting from an attractive influence exerted by the vitelline filaments, which, however, are presumably of the same nature as the central mass of the aster, and ought therefore to operate in the same manner on nuclear substance. The view that the equatorial accumulation might be due to the mutual attraction of the elements composing the nuclear disk, does not help to explain cases where (Limax, Figs. 86–89) the segmentation spindle lies far to one side of the pronuclei. Any scheme which admits that the substance forming the equatorial thickenings remains unaltered as regards its attraction or repulsion for other constituents of the egg, encounters the fact that this substance moves during successive periods in practically opposite directions. If there were any means of making it probable that a change in the (electric or other) conditions of the nuclear substance itself takes place while it tarries in the equator of the spindle, its movements might then be explainable as the result of the uninterrupted action of a single polar force, without involving the necessity of an entire reversal in the operation of the hypothetical influence. But for the present I see no way of accounting for a change in the nature of the moving *substance* which is any more satisfactory than the assumption of a reversal of the moving *force*.

There is one feature in the migration of the lateral halves of the equatorial thickenings which has, I believe, never been called in question by any of the observers who have studied objects in which the migration could be readily observed in the living cell. The separation is at first rapid, but subsequently the rate of the movement diminishes. The bearing of this fact on the location of the forces which induce the separation has not, so far as I recall, been stated by any one. It has more generally been held that the separation is due to a traction emanating from the centres of the asters, which draws asunder the halves of each of these thickenings. Others have assumed that it was the result of the mutual repulsion of the halves. So far as the rate of the separation is concerned, it appears to me to support the latter view; for, assuming, as is most natural, that there is no rapid change in the intensity with which the force acts, a diminution in the effect is what must necessarily ensue with a constantly increasing distance between the source of the force and the object moved. A gradually accelerated motion must, on

the other hand, be anticipated if the moving body is approaching the source of the moving power. But if this should be proved inconsistent with other phenomena accompanying nuclear division, it might be possible to refer the retardation to an increasing opposition to motion offered by the substance in the vicinity of the astral centre.

The slender threads which remain behind the separating lateral zones of thickenings, I have called, not to prejudge in the use of a name, *interzonal filaments.* If the spindle fibres are, as has been generally maintained, composed exclusively of nuclear substance, it is not apparent what these interzonal filaments may be. It has been believed by some observers that they represented a kind of product of the activity of the nuclear substance of the fibres. It has been claimed that their substance, like that of the thickenings, is ultimately employed in the growth of the new nuclei, and that consequently they are nuclear substance. That they differ from the spindle fibres and the varicosities is shown by their not staining as intensely as the latter, and by the fact that they do not as promptly respond to the force which causes the migration of the stainable substance. While the evidence is too strong to allow a doubt as to their being partly consumed in the growth of the new nuclei, I think there is sufficient proof that *the whole of their substance is not incorporated in the nuclei.* The evidence in Limax (Figs. 29, 80^a, and 91) is as satisfactory as could be expected in cases where the changes cannot be directly observed. Neither their deportment with reagents * nor their fate compels the belief that they are nuclear substance. I am therefore disposed to believe that they are in composition like the interstellate rays at their inception, — that they are, in other words, the spindle fibres deprived of their nuclear substance, and that they differ from the vitelline protoplasm with which they ultimately coalesce only in their greater compactness and refringency.

ORIGIN OF NUCLEI. — The formation of nuclei in early stages of ontogeny results from a fusion of nuclear substance with protoplasm, and occurs under two slightly modified forms. The production of the female pronucleus is like that which takes place at each segmentation, and offers no peculiarities capable of supplementing the knowledge of the process which one may acquire from ordinary cell division; but the origin of the male pronucleus occurs under such different circumstances, that the method of its formation throws additional light on the nature of nuclear production.

* Compare Flemming's statement, p. 360.

As Auerbach first pointed out, the new nuclei in division arise in the handle of the "dumb-bell." The early observers who make the centre of the aster the seat of the forming nucleus are unquestionably in error, even if it must ultimately be granted that the "areal corpuscle" at the centre of the aster forms subsequently an element in the new nuclear structure. This corpuscle certainly cannot be looked upon as the beginning of the new nucleus, which at an early stage lies at a comparatively great distance from it. It has been shown by the observations of Bütschli and Strasburger, and still more satisfactorily by those of O. Hertwig, that the new nuclei arise directly from the lateral zones of fibre thickenings, which, in turn, owe their existence to a rearrangement of nuclear substance. My observations serve to confirm this for Limax.

How the metamorphosis of the lateral zones is effected has not been so definitely established. Bütschli has claimed that the new nucleus begins by the formation of a very small, clear, fluid-filled space around the dark granules of each zone, and that the granules become the *nucleoli* of the new nucleus. Certain features of the metamorphosis in Limax seem to favor this view. A comparison of Fig. 90, where the first evidences of a segmentation furrow are visible, with Fig. 93, where the cleavage is only about half completed, shows that the changes in the nascent nucleus must be at this period very rapid. The existence of a large number of nucleoli in the second case is perhaps indicative of a direct genetic connection between the "thickenings" and the nucleoli, rather than a more radical metamorphosis out of which the nucleoli have arisen as new formations; and yet, in any event, time enough has elapsed for a considerable increase of the nuclear mass, as the size of the new nuclei clearly shows. In later segmentation stages, especially during the second cleavage, I have more satisfactorily observed the early condition of the nucleus, — most distinctly after treatment with osmic acid and Beale's carmine. When of about the size of the lateral zone in Fig. 90, it appears as a small uniformly stained *homogeneous* body. The pronuclei (Fig. 70^b) are also sometimes encountered in a homogeneous condition. But the rarity with which these stages are found lead me to think they are of exceedingly brief duration.

It is generally conceded that the nucleus is composed of at least two substances, which present properties different from each other and from the protoplasm of the cell. They have been designated by R. Hertwig as "Kernsubstanz" and "Kernsaft." O. Hertwig has explained the formation of the new nucleus as due to a process the reverse of that which takes place at the formation of the rods composing the middle zone of

thickenings. Whereas in the latter case there is a severing of these two constituents of the nucleus, in the former the nuclear substance, still in the form of rods, imbibes nuclear fluid, and the individual rods, swollen into granules, then become confluent, and thus is restored a nuclear mass of uniformly mingled constituents. So far as my observations extend, they do not directly conflict with Hertwig's views; but, theoretically considered, it seems difficult to explain what the significance of all this metamorphosis may be, if the same fluid constituents of the old nucleus are to be reabsorbed by the accumulations of readjusted nuclear substance. It appears to me much more reasonable to assume that that which is appropriated by the lateral zones is new substance from the neighboring protoplasm, and even not exclusively the more fluid constituents of the latter. If Hertwig's statements do not imply the reabsorption of the nuclear fluid set free at the disappearance of the membrane of the old nucleus, then I can accept his interpretation; for he says of a somewhat later stage, to explain the growth, that the nucleus possesses the ability to appropriate from the yolk "flüssige und feste Stoffe." But what may be said of the young nucleus in this respect may also be reasonably ascribed to the nuclear substance existing in the rodlike form. It appears to me certain, from the increase in the total mass of the nuclei with successive segmentations, together with the absence of evidence that the *proportion* of "nuclear substance" is correspondingly diminished, that the nuclear substance of the "thickenings," and afterwards the young nuclei, possess the ability to incorporate with themselves, not only the more fluid constituents from the yolk, which may represent the "Kernsaft," but also less fluid portions, which with equal propriety may be considered "Kernsubstanz." That the central areas of the asters, when such exist, sustain an intermediary relation between the protoplasm on the one hand, and the growing nucleus on the other, can hardly be questioned. It is a significant fact, that the fusion of these zonal rods into a homogeneous body only takes place when the latter have reached, not the apex of the spindle, but the edge of the "area," and are thus in a situation to avail themselves directly of the areal substance. Whether the latter is unaltered protoplasm, or whether it is protoplasmic substance which has already undergone changes rendering it more like that with which it is about to be incorporated, is not to be answered categorically; but the signs of chemical activity developing a force which affects the remotest portions of the vitellus are indicative of fundamental changes in the region of these areas, and the properties of their substance which have been observed

by Whitman (his "nucleoplasm") and others point in the same direction.

O. Hertwig has shown experimentally in the starfish, that, when fecundation is introduced before the formation of the female pronucleus, the male pronucleus attains the same dimensions as the female; but when fecundation is delayed until after the female pronucleus is developed, it remains much smaller than the latter. The explanation implied in Hertwig's statements is, that in the latter case *the female pronucleus has already appropriated*, as it were, *the whole of the available nuclear fluid.* The same theory Hertwig thinks valid in explaining constant differences in the relative sizes of the pronuclei after normal fecundation in different animals. Thus in Toxopneustes, where the events of maturation transpire before fecundation, the male pronucleus remains small, while in mollusks, etc., spermatization having been effected before these events, the two pronuclear bodies attain the same size. In so far, then, as this theory serves to explain phenomena, it establishes its claim to acceptance. But there exist certain objections to this view. It seems to necessitate the belief in a fixed amount of unengaged nuclear fluid, which, I believe it is fair to assume, the author must identify with that which was liberated at the metamorphosis of the germinative vesicle. That being the case, the theory necessitates the *uniform* diffusion of this liberated fluid through the whole yolk; as, otherwise, how could a male pronucleus, arising indifferently at any point near the surface, enjoy the same opportunity for the acquisition of it as a female pronucleus originating in the immediate vicinity of the place where it was set free? Although the male pronucleus has been seen before the formation of the first polar cell, it does not appear that it increases much in size, or is far removed from the surface of the yolk, before the production of the second polar cell; that is, before the time the substance of the female pronucleus loses its connection with the substance of the last polar cell. It then exhibits a more or less rapid growth and migration. But if it is simply dependent for its increase in size on the liberation of nuclear fluid, I see no reason why it should not greatly increase, even before the production of the first polar cell, in cases where the spermatization is effected before the events of maturation. And if Hertwig's reasoning is correct, why should it not, in these instances of early fertilization, acquire the major portion of the available nuclear fluid, and thus surpass in size the female pronucleus, instead of simply reaching an equality with it? May it not be that differences in the growth of the male pronucleus are explainable without recourse to the supposition of a fixed

amount of nuclear fluid? Its increase in size appears to be intimately connected with its migration, and the migration is apparently in response to the direct attractive influence of the female pronucleus, with which it is ultimately fused. In cases where the latter has attained its full size, its power of attraction will be greater, as its mass is greater, than at any earlier period in its growth, and the migration of the male pronucleus will be correspondingly more rapid, so that it will have less time to incorporate with itself substances from the protoplasm. It will reach the female pronucleus before it has acquired its normal size; but this it *may* subsequently attain by continuing to grow after encountering the latter, as Selenka has shown to be the case in Toxopneustes. This may perhaps explain the experimental cases, as well as that of Toxopneustes, where the female pronucleus takes a position in the centre of the yolk. It cannot, however, be claimed that migration and growth stand in the relation of cause and effect, since in certain cases (eccentric female pronucleus), when the male pronucleus arises near the animal pole, the extent of its migration is more limited than when it makes its appearance near the opposite pole; and yet it attains in both cases the same dimensions, and probably grows with the same rapidity. It can be said that both migration and growth appear to depend on the existence of certain conditions which are established with the elimination of the substance of the polar globules, but not that those conditions are fulfilled by the liberation of nuclear fluid at the time of the conversion of the germinative vesicle into an amphiaster; for the observations of Whitman on the "quiescent state" of the egg in Clepsine seem to afford the most satisfactory proof that the metamorphosis of the vesicle may transpire long before the enlargement of the male pronucleus, and nearly all observers concur in the statement that the two pronuclei arise at nearly the same time in cases where spermatization has preceded maturation.* From this it appears to me that the growth of the male aster, instead of corroborating, offers serious obstacles to the acceptance of Hertwig's explanation.

* From the evident dependence of the migration and growth of the male pronucleus on the existence of a more or less developed female pronucleus, it appears reasonable that with the detachment of the polar cells the character of the nuclear substance is so far altered that it exerts on the male pronucleus an attraction of which it was incapable when still joined to the nuclear substance that is removed with the polar cells. Whether this implies a greater difference than exists between the halves of the nuclear plate in ordinary cases of cell division, may be questioned. It should at least be remembered that there are in all cases indications of a mutual repulsion between the lateral zones of fibre thickenings.

There are probably at least two modifications of the process by which the fibre thickenings are converted into a single nuclear structure. It has been repeatedly shown, that in some instances the individual thickenings pass through a vacuolar stage, and that nucleolar bodies are found in the vacuoles *before* their ultimate confluence, — the "multinuclear" condition of the cell. In Limax I have been unable to detect such a condition, and am therefore inclined to believe that the differentiation of nucleoli does not take place until after the fusion of the thickenings. But this difference is not one of fundamental significance, since in cases where clusters of nuclei are developed their confluence in some instances regularly ensues much earlier than in others. Limax, then, only furnishes one of the extreme examples, since here the confluence takes place before the formation of nucleoli. The postponement of the fusion, observed in so many cases, and the consequent presence of a number of apparently unconnected vacuolar structures, no more warrant the conclusion that a *multinuclear* condition exists than does the earlier state, when the nuclear substance consists of a group of more numerous fibre thickenings. It is only a stage in the process of concentration into a single nucleus, and these different phases under which it occurs only serve to make this interpretation more reasonable. In all cases of a spindle differentiation the processes are essentially the same; there is a fusion of the nuclear substance of the thickenings with the protoplasm of the yolk, and the end result is a single nucleus.

The proportion of the nuclear substance from the old nucleus, which, by means of the "thickenings," directly contributes to the formation of the new nuclei, although approximately constant for a given stage in the development of any given animal, is subject to wide variations from one animal to another; and there exist even more extreme modification, between remote cell-generations in the same individual. In Limax, where the nuclei attain a large size, the proportion is very small, especially in the formation of the male pronucleus, if, as is probable, the latter is initiated by a single spermatozoön; somewhat greater in the starfish and the Hirudinea, for example, where the nuclei remain comparatively small. But in plant cells the proportion is often very large, and in certain tissue cells, as Flemming has shown, almost a maximum.

Whether there exist cases in which the old nucleus simply divides without any metamorphosis, — without the least interchange of substance with the surrounding protoplasm, — and, if so, whether the conditions prevailing in tissue cells present stages of transition between the more direct and the more complicated methods of nuclear division, —

can be satisfactorily answered only by further and extensive comparative studies. There are already many important observations which make such a direct division (without fibrous differentiation) probable; but even in such cases an interchange of substance may not be completely excluded, and the certainty that there is an increase in the total amount of nuclear substance with successive generations makes the acquisition of new material on the part of the nucleus unquestionable. That this acquisition is facilitated by the division may at least be claimed as probable.

It is extremely doubtful whether new nuclei arise in animal cells without the least visible connection with the nuclear substance of preexisting nuclei. Even in plant cells this process may be less certain than has been claimed. I have endeavored to show how the case of Isoëtes might possibly be less indicative of this mode of formation than Strasburger teaches; but the evidence from the conifers and from Phaseolus seems at present capable of no other interpretation than that there is often a complete dissolution — a morphological obliteration — of the old nucleus. It perhaps is not entirely unreasonable to indulge the hope that even in these cases new methods of investigation may ultimately prove that there is not an entire dissipation of the substance of the old nucleus. In either event, however, the interpretation which Strasburger has given is that which most fully explains the extreme cases.

Germinative Vesicle. — It has been conclusively shown both by Fol and by O. Hertwig, that the first maturation spindle is formed at the expense of constituents of the germinative vesicle. The latter is neither totally dissolved in the yolk nor totally eliminated from the egg. I have not proved the same to be the case in Limax; but there is no occasion to doubt that the first archiamphiaster is there produced in the same manner as in the starfish. So much being granted, there is every reason to agree with Hertwig that a genetic connection exists between the germinative vesicle and subsequent generations of nuclei. I have never found an egg which did not, under proper treatment, exhibit some definite morphological evidence of the existence of a nuclear structure, and am certain that in no case is the formation of the polar globules accompanied by an elimination of the whole of the spindle. The female pronucleus is formed primarily from the inner half of the nuclear plate of the second maturation spindle, and its substance enters into the composition of the first cleavage spindle. The evidence of the continuity

of the substance of the germinative vesicle with that of the nucleus of the first segmentation sphere, is as complete as could be expected of eggs which do not allow the demonstration of the spindle nucleus in the living condition. I have been led to suspect that certain phases of the metamorphosis — between the first and second maturation spindles — have not yet been discovered; but I have no evidence that such a hypothetical stage renders this continuity any less certain. On the contrary, if such an intermediate stage as I have suggested really exists, it can have no other effect than to remove the nucleus of the first cleavage sphere one generation further from the germinative vesicle, but does not even warrant the supposition that the former contains a smaller proportion of the nuclear substance of the vesicle than it would have contained had the course of events been such as recent observers, who admit the continuity in question, have claimed. But it is not a question of the *amount* of nuclear substance which thus finds its way into the nuclei of successive generations. The continuity is definitely and adequately established by the fact that generation after generation the nuclei have for their beginnings portions of the substance composing the nuclei of the stage preceding. There is not the least doubt that such beginnings exist in the lateral zones of fibre thickenings. The stage in the process which is least satisfactorily understood is that of the origin of the equatorial zone. There can be little question that it is formed in the same manner in all the earlier embryonic stages at least, and it is equally evident that it must in some way be formed at the expense of the disappearing nucleus. My own observations (Figs. 85–89) support this view in a decided manner. Whether, however, any portions of the old nucleus — and, if so, which — go over bodily and unaltered into this forming disk, seems much less certain. Hertwig's studies assuredly offer the best evidence we have that such is the case; but Hertwig has failed to demonstrate that the beaded elements of the nucleolus are directly incorporated in the equatorial thickenings. He has traced them as far as the central area of a forming aster, but that is not the equator of an amphiaster. His observations only strengthen his position that it is the nucleolus (and therefore "nuclear substance") which is immediately concerned in the building up of the nuclear spindle, and do not necessarily prove that it persists in the shape of fibre thickenings. I have once seen appearances (Fig. 85) which suggest that the substance of these thickenings may be aggregated into definite visible corpuscles in the region of the nascent spindle, independently of the fibres to which they ultimately belong; but I place little confidence in

this observation, and prefer to rest in the tentative belief that the thickenings are produced solely by molecular rearrangements of the nuclear substance, which is accumulated along the protoplasmic axis of the fibres. This view is not to be confounded with Auerbach's *karyolysis*, for it does not involve a dispersion of the substance of the nucleus through the neighboring protoplasm, and its re-collection. It implies a direct transfer of substance, but in elements too small to be individually visible even with the best optical aids.

Polar Globules. — The formation of polar globules has been shown to be a constant feature in the maturation of eggs in representatives of a majority of the recognized groups of animals. It is principally in such as are characterized by the possession of a large proportion of nutritive substance that their presence has not been established. The increasing evidence of their constant production warrants the assumption that they will be discovered in many cases, perhaps even in the larger groups, where they have not yet been seen. The great probability of their formation in Tunicata has been shown from the figures given by Strasburger.* Still, the thoroughness with which some groups have been

* P. S. — Grobben (**'79**, p. 209) has shown that in one of the Cladocera (Moina) there is imbedded in the yolk at the animal pole of recently excluded eggs a body which stains intensely in carmine. He believes it is a polar globule, which in this case has not been detached from the yolk. This is perhaps the best evidence yet produced to prove the existence of polar globules in Crustacea, and with further study may possibly serve to show some of the regressive steps by which their production has sunk from the dignity of cell division to a simple elimination of an amorphous mass at the primary pole of the yolk. I would call attention, however, to the possibility of a close relationship between this body and the polar accumulation of stainable substance (polar ring) described by Whitman in Clepsine. The indistinctness of its limitation from the yolk (*loc. cit.*, Fig. 3), and its not protruding above the surface of the same, are points of resemblance with the polar rings; but its persistence in late stages of segmentation (Figs. 5, 6) seems at first to indicate a different fate from that of the ring substance. But when there are fifteen blastomeres, the small sphere which contains the "Richtungskörper" is divided by an equatorial plane into two spheres of equal size, and Grobben adds (p. 212): "Mit dieser Theilung verschwindet der Richtungskörper von der Oberfläche des Eies, *da er offenbar in die Tiefe der oberen Furchungskugeln gelangt.*"

This seems to afford strong indications of the identity suggested, and, if once established, may remove Grobben's doubt as to the polar-globule nature of the bodies described by Leydig (**'60**, p. 145) for Daphnia longispina as "einige blasse Kügelchen, ganz vom Charakter jener unter dem Namen 'Richtungsbläschen' beschriebenen Gebilde," which he saw appear "an dem einen Pol ausserhalb der Eischale." It is noticeable that at the first segmentation this body (Grobben, Fig. 2) remains in con-

studied with this point in view, and especially the presence of conditions at maturation which appear to be, if at all comparable with, at least fundamental modifications of this process, seem to preclude the existence of typical polar globules in a number of the groups of animals, while the failure to find equivalents of the "canal cells" in the higher phanerogams is possibly even a greater obstacle to the claim that they are of universal occurrence.

The fact that it is the eggs possessing a large proportion of nutritive substance which deviate from the typical formation of polar globules would indicate that it might be the accumulation of this food material which interferes with the normal or more primitive method of maturation, and prevents the formation of cell-like polar bodies. There seems little doubt that the elimination of portions of the substance of the germinative vesicle as described by Balfour for Elasmobranchs, by Oellacher for bony fishes * and birds, and by Van Bambeke and Hertwig for Amphibia, represents in some hitherto unexplained manner the formation of polar globules. It is perhaps safe to indulge the expectation that some of the representatives of these groups will ultimately furnish the means of explaining how the two processes are reconcilable: at present it does not seem possible to present a satisfactory hypothesis of their mutual relationship. It can only be said that in all cases there is probably an elimination of a part, and of only a part, of the substance of the germinative vesicle together with a small portion of

nection with the blastomere, which appears to be a trifle the larger, — just as in Clepsine the oral-ring substance does (compare Whitman **'78**[a], Fig. 15), — and which takes the lead in the production of the small cells about the primary pole, exactly as Whitman (Figs. 19, 20) has shown to be the case in the leech. That the ringlike disposition of the substance is in no way a necessary feature follows from the condition (Whitman, Fig. 70) presented by the "aboral ring." This, however, is no argument for the identity of Grobben's body with polar rings, since the polar globules sustain in Clepsine the same relation to the blastomere which leads in segmentation.

Henneguy (**'80**) has also reported the discovery of polar globules in one of the Crustacea (Oniscus).

* P. S. — Hoffmann (**'80**) has given a preliminary account of the early stages of several osseous fishes, in which he shows that a single polar globule is produced in the normal way from the external half of a maturation spindle. He claims, however, that, as the nucleus which is formed from the inner half of this spindle is the "Eikern," so the nucleus which is formed from the external half is the "Richtungskörperchen." Can it be that this represents a transition from a process where the production of polar cells entails the loss of a certain portion of the yolk to one where there is an elimination of *only nuclear* material, or is it to be assumed that Hoffmann's statement is, from its brevity, slightly inexact?

the vitellus. In the case of mammals there is sufficient evidence of the existence of polar globules, as the early studies of Bischoff indicate, but no one seems to have yet discovered the method of their production, and it is therefore open to question whether they arise by a process of cell division from the external halves of maturation spindles. Ed. van Beneden's account for the rabbit does not remove the uncertainty.

There are, besides, two other groups of animals in which the presence of anything even remotely comparable to polar globules has not yet been satisfactorily determined, — Rotifera and Arthropoda. Flemming's expectations in regard to the existence of the polar globule in Lacinularia were not confirmed by Bütschli, who directed particular attention toward their discovery in the Rotifera. The accounts of their formation in certain Crustacea * also need further confirmation.

The polar globules may be considered from three standpoints, — the morphological, the physiological, and the historic, or phylogenetic.

Morphologically viewed, there can no longer be any doubt that they are *cells*. They are formed by a process in all essentials like ordinary cell division. They are composed of a protoplasmic substance which stains feebly, and of a nuclear substance which stains deeply. The latter is derived, through the intervention of a fibrous spindle and a dividing nuclear plate, from the nuclear substance of the immature ovum. The lateral zone of thickenings in the globule is not always massed into a *single* nuclear structure. It is possible that in many cases this condition is exhibited because sufficient time has not elapsed for the accomplishment of the successive acts of the consolidation. But in any event the conclusion seems inevitable that there is a decline in the functional activities of this cell, which delays the completion of its work beyond the normal period of such changes. It is probable that many polar cells never would have attained this typical condition, — a cell with a single nuclear structure, — even if their activities had not been intercepted by the action of reagents. A decline in the functional potency of the polar cell is ultimately followed by a complete surrender of its morphological integrity. That, however, does not warrant a denial of its morphological value as a cell, any more than the gradual obliteration of the structure of an element from the epidermis would justify a denial of its cell character.

Notwithstanding the cell nature of the polar globule, there is one morphological peculiarity connected with its production, besides its diminutive size, which has been previously observed, it is true, but which has

* Consult Leydig '60, p. 145; Dieck '74; and Hoek '76, p. 62.

not been sufficiently emphasized. This unique feature is *the coalescence of the "areal corpuscle" of the external aster with the envelope of the polar cell at its distal extremity.* It is a peculiarity which may perhaps be of importance in two ways. It is possible that it will some time help to a better understanding of the forces at work in the process of cell division, and it may also be of importance in deciding what share the "areal corpuscles" of the stellar figures take in the formation of new nuclei. O. Hertwig is, so far as I am aware, the only observer who has given any representation of such conditions as I find in Limax. In the cases especially of Nephelis and Hæmopis he ('77, Taf. I., II.) has figured the tip of the spindle as lying at the surface of the egg, and he mentions (p. 20) "ein dunkles Korn, die *peripher* gelegene Spitze der Spindel." The figure referred to in this connection (Taf. II. Fig. 3) is perhaps in this particular the least satisfactory of all those given by Hertwig; for the "Korn," although represented as being "peripheral," is not in contact with the outline of the polar globule. If the apex of the globule were turned a little toward the observer, so as not to be seen exactly in meridional section, the "Korn" might appear, as it does in his figure, at some distance from the surface (i. e. from the outline), even if fused with it. One hardly has the right to assume that so skilled an observer could have mistaken the position of this conspicuous granule; otherwise I should conclude that in this case, as in a majority of the eggs represented, the tip of the spindle (its "Korn") was merged in the envelope of the polar cell. That such is really the case in Limax I have not the least doubt. Evidently, then, *in the polar cell, the "areal corpuscle" takes no part in the formation of the new nucleus.* The question naturally arises, In how far are the conditions realized in the polar cell duplicated in the egg cell? If the areal corpuscle contains nuclear matter, and is essential to the completion of the new nucleus, how can it so completely fail to realize its true destiny in the polar cells? Although I can consider the question hardly more than fairly stated, still there are some features in the formation of new nuclei, already discussed, as well as certain facts concerning the place where these "areal corpuscles" first appear, which lead me to think that it may not be necessary to interpret the latter as nuclear substance. Such a view might cause this peculiarity of the polar cells to appear less bizarre. The relation of the centre of the external aster to the envelope of the polar cell is certainly unlike its relation to that of segment spheres in ordinary cell division; for there is no case, however small one of the products of segmentation, in which the centre of the aster approxi-

mates the outer wall of its cell. This is a peculiarity in the formation of polar cells which deserves more attention than it has received. It may perhaps be urged as an indication, that the production of polar globules is not accomplished by cell division; but it appears to me that it does not present any fundamental obstacle to that conception.

In an examination into the nature of the forces which result either in the production of the polar globule or in cell division, this peculiarity may furnish some means of extending or correcting conclusions to be drawn from less modified forms of division.

No theory of a mutual repulsion between the stars of the amphiaster is able to explain either the "orientation" of the spindle, or its migration to the surface, nor is the existence of such a repulsion at this stage certain, since the length of the spindle (with a single exception, noticed later) remains during this period practically unchanged.* Neither does it seem possible to explain the migration as due to the attraction which the centres are supposed to exercise on the vitelline protoplasm, even if such an attraction were capable of putting the spindle in a central position in reference to the active constituents of the vitellus, or of causing it to occupy the primitive axis of the yolk. No such attraction could urge *both* asters into such close approximation to the animal pole. There may be complicated chemical and physical processes underlying all the movements connected with the formation of polar globules which are at present as unintelligible as are all other spontaneous movements of protoplasm. Still it is not useless to inquire whether there is any possible explanation of the movements of the archiamphiaster which, without dealing with the nature of protoplasmic motion in general, is capable of rendering these changes less obscure. An assumed attraction, exerted by the protaplasm upon certain constituents of the spindle (the inner half), and a repulsion of other constituents (external half), would be sufficient to cause the "orientation" of the spindle in the primitive axis; but a migration which carries its internal end beyond the centre of the active protoplasm toward the primary pole could only be accomplished by the repulsion preponderating over the attraction, and even with that assumption a lengthening of the spindle should result from the repulsion of one of its ends and the attraction of the other. The final separation of the repelled portion, accomplished by the formation of polar globules, would then leave the

* In the *early stages* of its formation a mutual recession of the asters has been observed, and may perhaps be attributed to the development of mutually repulsive properties, but after the formation of the spindle there is no important separation.

attracted portion free to move in the direction of the centre of the attracting protoplasm, be that the centre of the yolk or a point nearer the animal pole. The case of the migration of the germinative vesicle before its constituents are converted into a spindle would demand no special modification of the assumed forces. The most serious objections to this explanation are the constancy of the distance between the asters during the migration and the entire similarity of the lateral nuclear plates, both in size and behavior, which appears incompatible with their being affected in different degrees by the assumed attraction and repulsion.

Simple contractions on the part of the vitellus might under certain circumstances produce the same result. If the yolk presented in its primary radius a structural condition which offered less resistance than other radii to the progress of a moving body, any contractions of the vitellus would cause the amphiaster to passively advance along this radius until it reached the surface, and finally cause its protrusion. That this is structurally different from other radii is sufficiently obvious in many cases, but I do not know that there is any direct evidence of the condition (more passable) assumed. This would make the whole amphiaster entirely passive as far as regards the migration, and it would afford no explanation of the cause which induces the *return* of the internal half of the spindle toward the centre of the egg as a female pronucleus. It may be of some importance in this connection to know just when this migration of the amphiaster takes place. Whitman has shown that a quiescent stage may intervene between the formation of the first archiamphiaster and that of the first polar globule; but I am not quite certain how far the egg has advanced when this interruption of activities is manifest. It seems most likely from his account that although the "polar figure" (which results from the presence of one of the asters near the surface) makes its appearance, the centre of the external aster does *not* reach the surface until after the egg emerges from its quiescent state. At least, he does not mention the existence of the "pellucid spot" at this stage, and states that it usually appears from ten to twenty-five minutes after the egg is deposited. It is therefore probable that the contact of the astral corpuscle with the limiting envelope of the egg takes place during the series of vitelline contractions which terminates in the production of a polar cell. These contractions have been shown to assume in Clepsine a most remarkable and uniform appearance, — a constriction advancing from the equator toward the primary pole. It is not to be claimed that the migration of

the spindle is accomplished directly by the movement of this wave-like constriction, for the pellucid spot is seen before the wave has begun to approach the primary pole of the egg. It is only probable that the migration is accomplished by contractions of the yolk, of which this is a special manifestation, and moves toward the animal pole, because that radius corresponds with the line of least resistance. Evidences of contraction are not so marked in Limax, but still they are exhibited in alterations of the general form of the egg, and especially in the constancy with which the primitive axis is shortened. (Compare also Fig. 55.)

But whether it be the result of a repulsion, due to physical or chemical conditions of the substances concerned, or simply of the contraction of the yolk, the amphiaster certainly appears in this movement to be more acted upon than acting. There must be some influence operating from behind to account for the deflection of the rays of the outer aster, and it is probably the same force which induces the shortening of the spindle observed by O. Hertwig in Asteracanthion just before the formation of the polar globule.

The physiological significations which have been attached to the polar globule have differed widely, from an important determining influence upon the course of subsequent events in segmentation to a meaningless exudation of liquid from the yolk. While its morphological place is well established, it does not of necessity follow that its function is explainable from the same data. It may not be as rational now to say that it is without meaning, as it was when Rathke pronounced that verdict; but its being a cell will not be found sufficient evidence that it is not the means of removing useless or undesirable material. It soon undergoes disintegration, and certainly has no further importance in the economy of the embryo: these have been brought forward as evidences to support this view. That its present functional importance consists only in the removal of certain substances from the egg receives further support from the continuance of the process of removal in cases (Batrachia, etc.) where the cell condition is no longer maintained. But an objection to any view which discovers in this phenomenon only the removal of worn-out material is that the removed substance partakes of the nature of the cell in every essential particular. It certainly embraces nuclear substance and more or less granular protoplasm. The changes which accompany its formation are, save in one or two minor points, like those which accompany ordinary cell division; its nuclear substance assumes the same conditions (though not so promptly)

as the nuclear substance in the remaining portion of the egg. There are some indications that its substance acts less vigorously than that of the larger cell, but it certainly has not lost all its reconstructive ability. It is therefore unlike any known products of secretion or defecation.

Bütschli has advocated the opinion that the principal physiological signification of the globules consists in the removal of a part of the "Eikern" (germinative vesicle); and Strasburger adopts the same view in saying that the nucleus frees itself of certain constituents, and thus makes ready for the approaching fecundation. Bütschli's opinion that this elimination is due to fecundation, or is at least a phase in the early development of the egg, not in its maturation, must be abandoned, since it has been shown that the globules are in many cases formed before the approach of spermatozoa. It seems, however, to be indicative of a mutual influence of spermatic and polar-globule substances, that the male pronucleus is retarded in its migration and growth up to the time of the detachment of the second globule, as though the presence of the polar-globule substance acted as a hindrance to its normal development.

Balfour has adopted nearly the same view as Bütschli. He explains the act as consisting in the removal of parts of the germinative vesicle, more or less *essential* to the further independent development of the cell, to make room for the supply of the necessary parts to it again by the spermatic nucleus. This hypothesis would serve, he thinks, to explain why it is that polar globules have not been found in those groups (Arthropoda and Rotifera) where parthenogenesis is most frequently encountered. The fact that parthenogenesis is possible where impregnation is the normal occurrence, may appear, he says, to be an objection; but it cannot be denied without further study that development in such cases may be due to the suppression of the globules, and that when they are formed development without impregnation is less possible.

An objection to this latter assumption is the tardiness in the events of maturation which must be admitted when fecundation is under the control of the parent. If there were a suppression of the polar globule in the case of unimpregnated eggs of the honey-bee, for example, then the first steps in the formation of the globule must normally take place *after* the egg has passed the seminal receptacle, for the fecundation or non-fecundation of any given egg is not previously determined. In other words, the development of the egg up to that epoch must be the same in all cases, whether a polar cell is to be formed or not. Such a delay in the events of maturation may not be impossible, but does not

appear very probable. If the polar globules exist simply to remove an *essential* substance in order that another essential element may take its place, the whole process would appear to be a waste of energies with which nature is not often chargeable, unless it can be shown that some serviceable end is reached by such an exchange.*

None of the physiological interpretations offers any explanation of the most characteristic feature of the polar globule, — its cell nature. Evidently, any theory to be entirely satisfactory must explain the significance of this fact. Even if it be granted that its present function is one that may be accomplished without its assuming the condition of a cell, it will be useless to attempt to elucidate its full meaning without recognizing the importance of that peculiarity. The constancy of this morphological characteristic points to one of two things; either there is some peculiarity in the present function of the globule, which is best subserved by a cell-like structure, or it is simply the heritage from a former state in which the polar cell may have had a different functional signification from that to which it now responds.

* P. S. — Balfour ('**80**, p. 63) suggests "*that the function of forming polar cells has been acquired by the ovum for the express purpose of preventing parthenogenesis.*" His reasons for this conclusion are stated as follows: "The explanation given by Mr. Darwin of the evil effects of self-fertilization, viz. the want of sufficient differentiation in the sexual elements, would apply with far greater force to cases of parthenogenesis."

"In the production of fresh individuals, two circumstances are obviously favorable to the species: (1.) That the maximum number possible of fresh individuals should be produced; (2.) That the individuals should be as vigorous as possible. Sexual differentiation (even in hermaphrodites) is clearly very inimical to the production of the maximum number of individuals. There can be little doubt that the ovum is potentially capable of developing *by itself* into a fresh individual, and therefore, unless *the absence* of sexual differentiation was very injurious to the vigor of the progeny, parthenogenesis would most certainly be a very constant occurrence; and, on the analogy of the arrangements in plants to prevent self-fertilization, we might expect to find some contrivance both in animals and in plants to prevent the ovum developing by itself without fertilization. If my view about the polar cells is correct, the formation of these bodies functions as such a contrivance."

Why the eliminated substance takes the form of a cell, still remains as difficult of explanation as before. But the principal obstacle to the acceptance of this hypothesis is that the *presence* of polar globules in the *fertilized* eggs of Arthropoda and Rotifera has not been satisfactorily established in a single instance, (compare, however, the statements made above concerning polar globules in Crustacea,) much less shown to be an event of common occurrence. There can be no urgent reason for claiming that there is an omission or suppression of an event not yet shown to have an existence.

So far as has yet been ascertained, this peculiarity has no *present* importance, and I know of nothing which affords the least ground for anticipating such a discovery.

Several observers have raised the question what may be its historic meaning. Rabl's theory of a cœnogenetic origin has been already considered. From a comparison with studies on conjugation among Infusoria, Bütschli has arrived at the conclusion that the globule has a palingenetic signification. The formation of polar globules is a part of the process of fecundation, and is equivalent to the elimination (mutual interchange) of "nucleoli" in the temporary conjugation of Infusoria, and the increasing evidence of their universal occurrence renders such an (historically) early origin the more probable. Certain objections to this interpretation, partly anticipated by Bütschli, have been emphasized by Whitman, who, besides, finds evidence of the reasonableness of another theory in the "absence of such cells (equivalents of polar globules) among the Infusoria." It may be said in addition, that the cell nature of the polar globule, being now definitely settled, precludes that strict comparison of its substance with the "secondary nucleus" of Infusoria to which a purely nuclear composition (as at first claimed by Bütschli) would have presented no obstacle.

The opinion, first defended by Strasburger, that the polar globules have their counterparts in the "canal cells" of plants, opened the way for the theory (compare p. 463) which Whitman has ably advocated. The polar globules are a relic of the primitive or asexual mode of reproduction. A gamic cell-generation is followed by a line of agamic generations, the last of which are the polar globules.

This is the only view which offers the least explanation of the fact that these globules are cells. I believe it forms an important step toward the solution of their meaning; but it does not explain why this agamic process of cell-proliferation reappears after a long period of quiet growth on the part of the ovum. The signification which most naturally suggests itself in this connection is that they are representatives of once functionally active ova; that the renewed proliferation was formerly a means of increasing the number of the reproductive parts, just as in the formation of the spermatozoa the mother cells, after a period of growth, finally break up into a number of individual elements. In the case of the male elements natural selection has operated, through the multiplied chances of their failing of the opportunity to execute their normal function, for the preservation of the functional integrity of every individual, and even for a great increase in the number of the elements which

arise by this last act of proliferation. In the case of the ova different influences have been in operation. The *vigor of the elements* has here — i. e. as compared with the male elements — been a more important factor in the preservation of the individual and the ultimate success of the race than the multiplication of numbers. The last act of proliferation has therefore never resulted, in this case, in the production of more than a very few individual elements, and these have practically been still further reduced by the suppression of the function of the cells called polar globules, in order to afford the remaining cell (ovum) that increased chance of survival which a better equipment is capable of insuring.

If this view is tenable, the polar globules are *rudimentary* structures, and, as such, would be likely to present the peculiarities of such parts.* There is, in fact, a considerable variation in the size of the globules in the same species, and a more conspicuous variability in the promptness with which the reconstruction of the lateral zones of fibre thickenings into nuclei is effected. Accepting Strasburger's conclusions as to the equivalency of polar globules and the "canal cells" in plants, the "Bauchkanalzelle" of cryptogams may perhaps afford even a more obvious instance of variability in size.

The most important recommendation which this view possesses is the explanation it offers of the morphological condition of the polar globules. It would also serve to explain the signification of the peculiar phenomena observed by Whitman to accompany the production of the globules in Clepsine. The formation of an equatorial constriction might then be viewed in the light of an atavistic tendency on the part of the cell to divide in the original manner into two ova of equal size, and the gradual, orderly shifting of the constriction as a rapid recapitulation of changes slowly realized in the history of the race.†

* "Rudimentary organs are very liable to vary in development and in other respects in the individuals of the same species. Moreover, in closely allied species, the degree to which the same organ has been reduced occasionally differs much." — Darwin, Origin of Species, (5th edit., London, 1869,) p. 538.

† P. S. — The cases of the penetration of a spermatozoön into a polar cell, reported by Fol ('79, p. 246), possibly afford further evidence that these cells are aborted ova.

APPENDIX.

Of the numerous papers bearing on the topics here considered which have come to hand too late to be reviewed in their proper connection, I shall give an account of only those of Fol, Mayzel, and Pérez ; — those of the two last-mentioned authors, because they are based upon the observation of animals very nearly related to Limax campestris; that of the first, because presenting features of general interest, as well as many theoretical considerations concerning matters which I have discussed.

Mayzel's ('79) paper is a brief notice of the nuclear metamorphosis during segmentation as observed in the case of nematodes and Limax variegatus. His observations were first made public at a session of the Warsaw Medical Society, Nov. 26, 1878.*

The author undertook these observations primarily with the object of ascertaining whether Auerbach's view of a *karyolysis* or Brandt's theory of an *amœboid* nuclear division best explained the phenomena. He therefore selected for study Ascaris nigrovenosa and Strongylus auricularis. It is only as accessory evidence that Limax is introduced, and there is no attempt made to present a connected history of the early stages of its development.

The best success was reached by the use of acetic acid, and of the nematodes Strongylus proved to be the more favorable for study by this process. Mayzel succeeded in demonstrating in the nematodes mentioned the existence of the typical fibrous Kernspindel, with granular equatorial Kernplatte, and fibrous rays around the poles of the spindle. The presence of a small spindle located at the periphery warrants the inference that the elimination of a polar globule is preceded by the formation of a maturation spindle.

In Limax the author also found the spindle, nuclear plate, and astral figures, which accompany the first cleavage. The spindle consisted of very numerous closely packed, smooth filaments ; the nuclear plate was composed of highly refractive granules of unequal size ; the astral figure, of fibres very similar to those of the spindle.† I can corroborate for the species I have studied the description he has given, but insist upon the ultimate development of a more prominent distinction between the spindle fibres and the stellar rays than he seems to have observed. These differences in the condition of the spindle fibres may

* See Mayzel '79[a], '79[b], and '79[c].

† "Ebenso sind die sonnenförmigen Figuren an den Polen der Kernspindel aus aüsserst zahlreichen, glatten und somit den Spindelfasern ganz ähnlichen, gleichfalls sicher wahrzunehmenden Fäserchen zusammengesetzt, bestehen mithin nicht aus in Reihen angeordneten Körnchen; letztere füllen zwar die Zwischenräume zwischen den Fasern aus, lassen sich aber durch Druck auf das Deckgläschen leicht herauspressen."

possibly be accounted for by the more or less advanced condition of the spindle formation. The same differences in the degree of advancement may also explain why Mayzel did not meet with the nuclear plate composed of granules of a more uniform size. The conditions he has described I have also seen; but I have besides that seen other conditions which do not warrant the conclusion that the spindle fibres *remain* like the astral rays, even though they may still be called smooth filaments.

There is one point especially in which my own observations have not the clearness I should desire. I have not been able to remove all doubt about the confluence of the lateral zones of fibre thickenings into a *homogeneous* nucleus. It has been difficult to exclude the possibility that in some cases the granulations may persist to form the nucleoli. Unfortunately, the observations of Mayzel do not serve to make the matter any more certain. He says: "Die aus 10–15 Kleinen, hellen ovalen und rundlichen kernähnlichen Gebilden bestehenden 'Kernhaufen,' welche ich im Sinne Bütschli's als zusammenfliessende deuten möchte, erscheinen bei der Isolation wie *von einer gemeinschaftlichen Membran umgeben.*" I am unable to reach an entirely satisfactory conclusion as to the nature of the bodies thus described by Mayzel. If, as the author thinks, they are identical with Bütschli's "cluster of nuclei," the signification of the surrounding membrane is not apparent. Bütschli has described no such structure, and each of his "Kernchen," moreover, contains one or more nucleolar bodies. These Mayzel does not mention, and I have never seen in Limax anything corresponding to this condition. It therefore appears more probable that the description given by Mayzel relates to a subsequent stage, — to one at least as late as that shown in Fig. 93, — and that his nuclear bodies are really *nucleoli.* But the homogeneous condition of the nucleus, if it exists at all, precedes this stage. It is certainly an objection to the view I have suggested that the author looks upon these nucleus-like structures as in process of fusion. That cannot be claimed, I think, for the 8–10 small structures shown in Fig. 93, since my preparations show a constant increase in their number accompanying the increase in the size of the nucleus. I find, then, no sufficient grounds in Mayzel's observations for changing the opinion previously expressed, that the thickenings of the lateral zones fuse into a *single homogeneous nucleus* at an early stage, namely, before the differentiation of nucleoli.

The researches of PÉREZ ('79) on presegmentation stages in Helix aspera possess the merit of covering the very early conditions of the egg, — the changes which transpire while it is still in the "diverticulum" and the oviduct, — but the general conclusions at which he arrives do not appear to me to offer so satisfactory an explanation of the phenomena as the more generally received opinion.*

* Pérez alludes to the eggs of Limax agrestis, simply to call attention to the fact that when immersed in water the shell becomes distended by the accumulation of water between it and the membrane of the albumen. This, he says, may perhaps explain why Van Beneden held that there was a special liquid between these two layers.

He has summarized his own results nearly as follows : —

1. The first sign of evolution seen in the mature egg just arrived in the diverticulum, where it is fecundated, is a peculiar cloudiness of the germinative spot and the appearance of two small " nucléoles " in it.

2. The spot becomes diffluent, and difficult to perceive. The germinative vesicle tends to dissolution.

3. In the protoplasm of the nearly or entirely vanished vesicle there appears a double star resulting from the liberation of the two " nucléoles " from the disintegrated spot. The first system of radiations is thus established.

4. Meanwhile protoplasmic expansions arise from the surface of the yolk. It is not easy to comprehend their import. After a time, they re-enter the vitellus.

5. The rays extend and promptly reach the limits of the clear space left by the vanished vesicle, and invade more or less the vitelline mass itself.

6. There always exist at the centres of radiation small nuclei (noyaux), — the enlarged " nucléoles " of the germinative spot.

7. When these nuclei have attained a certain size and a vesicular wall, their vital energy, as well as the attraction which they exert on the surrounding protoplasm, diminishes.

8. The radiate substance then loses its consistency, becomes more fluid, and is expelled in two successive drops by the pressure of the surrounding vitellus. Thus the two polar globules are formed.

9. Neither the stars nor the spindle as such take a direct part in the formation of the polar globules.

10. These bodies elevate at the surface of the yolk a fine membrane, thus demonstrating that a vitelline membrane exists.

11. After the formation of the polar globules the double system of radiations is no longer present ; the two nuclei previously located at their centres lie in the yolk destitute of " aureola." Their volume has increased.

12. Since these nuclei have the same origin, directly from the germinative spot, one of them cannot be considered as a spermatic nucleus.

13. The two nuclei continue to grow, and their " nucléoles " subdivide by an irregular cleavage until reduced to a multitude of fine granules.

14. The conjugation of the two nuclei is far from being demonstrated.

15. It is more probable that one of the two is totally destroyed, and that the other persists as vitelline nucleus, to give rise to two " nucléoles," which become the centres of a new radial system embracing the whole extent of the vitelline mass, and determining the segmentation.

It will be seen that, although basing our conclusions on the study of very nearly related mollusks, we are in agreement in few particulars. This may in part be explained, I think, by the fact that Pérez seems to have made very limited use of reagents, especially in certain stages.

As I have not traced the origin of the first archiamphiaster, it will perhaps appear out of place for me to offer any criticism of the account given by Pérez. If, however, it be granted that the stars of the first segmentation am-

phiaster arise in substantially the same manner, — a thing which no one will be inclined to question, and which Pérez has himself assumed (pp. 392, 393) to be true, — then the observations on Limax have an important bearing on the point in question. I have shown that the stars arise not only outside the nucleoli, but even outside the nuclei, at a time when there is no evidence of an interruption in the continuity of the nuclear membrane. It is therefore impossible to ascribe the stars to the attractive influence of any morphologically distinguishable portion of nucleus or nucleolus. If any part of the substance previously contained in the nucleus occupies the centre of the stars, it must have suffered changes entirely incompatible with the retention of its morphological integrity.

My confidence in the real equivalency of the astral phenomena throughout the animal kingdom is too strong to allow the belief that the eggs of Helix differ in such essential matters from those of Limax. I am therefore compelled to seek an explanation of the author's observations which shall be less at variance with evidence drawn from the study of other animals.

He says that there always exist at the centres of the radiations small nuclei, the grown-up nucleoli of the germinative spot. His own observations and figures, however, do not warrant the assertion.* Of the three figures (Figs. 16, 17, 22) which he gives of amphiasters only one shows any central corpuscle, and only one of the asters of that amphiaster is thus furnished. Figs. 15 and 18, in which such structures are shown, are capable of a very different interpretation from that given by Pérez. But that is of less importance than to know whether these corpuscles are derived directly from the germinative spot. I therefore give the substance of the author's more extended account of the metamorphosis. "La vésicule [tache ?] germinative, d'abord uniformément brillante (Fig. 6), perd son homogénéité ; elle se trouble, sans pourtant cesser d'être claire, par la production de granulations pâles et mal limitées (Fig. 7). Au milieu de ces granulations, on distingue ordinairement assez bien deux petits nucléoles, que les réactifs rendent plus évidents. Ce sont là les premiers signes de désorganisation de la tache." (p. 364.) Subsequently, as though undergoing dissolution, it becomes pale, and is recognized with difficulty. Meanwhile the germinative vesicle loses its sharp contour, its membrane becomes folded, and an instant later has entirely disappeared. Then there remains only the protoplasm which it contained and the remnants of the disintegrated

* When he speaks (p. 395) as though it were a matter of surprise that these corpuscles had not been recognized as existing in all cases at the centres of the asters, one is not quite certain what can be meant. They certainly have long since been seen, and have been recognized as occupying the centres of the asters. To cite a single case, Flemming (**'75**, pp. 120, 191, Taf. III. Fig. 2) has shown that they exist in Anodonta, and has moreover called attention to their deportment under the influence of reagents. He has also stated that they are not constantly present. So far as I can judge, Pérez has not *always* found them, and my own observations seem to corroborate the fact of their inconstancy. He has seen these corpuscles in the egg before treatment with reagents. I have not been so successful.

"spot." The vitelline granules invade more or less this fluid substance, and thus diminish the size of the clear space. "Presque aussitôt se manifeste dans cet espace un système radiaire, ... qui consiste en un corps fusiforme aux extrémités duquel se voient deux soleils. Comment cette formation a-t-elle pris naissance ? Pas plus que les auteurs qui m'ont précédé, je n'ai vu naître sous mes yeux ce système de radiations." Notwithstanding a careful study of fresh eggs in which the germinative spot was in a state of dissolution, no conclusion was reached by Pérez, except that this stage is of short duration. Acetic acid, however, has sometimes shown in the germinative vesicle on the point of dissolution a pale and ill-defined double sun in the midst of irregular fragments coming from the spot (Fig. 8). "A slight pressure easily destroys this system, which I have never seen displayed in eggs whose germinative spot, less disintegrated, still allowed one to see its two nucleoli."

It is clear from the foregoing that the supposed identity of the two "nucléoles" with the corpuscles of the asters rests entirely on the negative evidence that the "nucléoles" were not to be seen after the appearance of the stars. It is not even shown that the corpuscles exist at the centres of the stars in the early stage represented in his Fig. 8 ; and there is nothing in this figure, unless it be the nearness of the two stars to each other, which is in the least inconsistent with their having originated *outside* the nucleus (germinative vesicle) ; for the outline of the latter is no longer visible, and the clear space shown is not of necessity due exclusively to the substance of the vesicle. But Pérez says further, that as long as the "nucléoles" are distinguishable in the spot, reagents only render them still more evident. When the spot becomes confused, however, either the reagents do not cause any definite form to appear (attributable to the fact that the objects are extremely fragile), or a double sun is seen. Less satisfactory evidence of a direct continuity could hardly have been presented. Confessedly there exists a stage in which, both before and after treatment with reagents, no definite structure is discernible. How, then, can it be possible for the "nucléoles" to persist as central corpuscles in the stars?

Pérez seems to have been equally unfortunate in tracing the further history of these corpuscles. He has often observed in the unaltered egg, either at the centre of one (Fig. 16) or of both suns, a very small nuclear body very slightly refringent, and surrounded at a little distance by a vesicular wall, — a body having, in a word, all the characters of a very young cellular element (p. 371), and he subsequently (p. 372) says distinctly that it is possible to obtain all the transitions from the condition presented in Fig. 16 (compare Fig. 48, *a a'*, of Limax) to the elements which are subsequently seen at the two extremities of the spindle ; but unfortunately he has not figured such intermediate stages. It is true that Figs. 15 and 18 are intended to show such intermediate conditions, but these I believe to be figures of a much later stage than is supposed by Pérez. He says (p. 400) that Fig. 15 is from an egg which had not yet produced the first polar globule. In that I think the author may be in error. He has said at p. 363 : "On peut trouver dans le diverticule d'une Hélice des

œufs génétiquement plus âgés que des œufs qui, chez une autre, sont arrivés dans l'oviducte, et déjà revêtus d'albumen. Il y a même plus : des œufs venant d'être pondus n'ont quelquefois pas atteint encore la phase de l'émission des globules polaires, alors que, chez d'autres Hélices, ces corpuscules s'observent déjà dans des œufs occupant le quatrième ou cinquième rang dans le haut de l'oviducte." All the phenomena which we have hitherto studied (i. e. up to the formation of the polar globules) may be observed, he says (p. 375), "in the egg contained in the diverticulum"; and a little farther on he adds, that Figs. 15 and 18 have been furnished by an egg from the oviduct. In view of the great variability thus described by him it certainly would not be impossible that an egg taken, as this was, from the oviduct, should have already produced the two polar globules. I have not the least doubt that that is what has transpired in the present instance, and consequently that the author has overlooked the existence of previously formed polar cells. If it were permitted from his descriptions to suppose that Fig. 18 was not from the same egg as Fig. 15, then the latter might possibly represent the first archiamphiaster, — a stage antecedent to the formation of polar globules; but in his explanations of the plates (see also p. 372) he describes Fig. 18 as "ce qui reste du corps radiaire de la figure 15, quand on a dégagé par de légers coups sur la lamelle la majeure partie de la substance qui cache les noyaux." Still the difference in the relative sizes of the nucleolar bodies in the two figures may possibly be taken as evidence that they were not drawn from the same egg, and that the author only intends in his "Explanations" to convey the idea that Figs. 15 and 18 are drawn from eggs of the same degree of advancement. However it may be with Fig. 15, Fig. 18 represents not the vesicular remnants of two astral figures, but the two *pronuclei* already closely approximated. It is more reasonable to suppose that Pérez has in this case overlooked the existence of the polar globules (a thing which may very naturally happen, since they are easily detached) than that all observers before him have overlooked the presence of a large vesicular structure embracing the central portion of each aster. He asserts that "such is the transparency of the two 'cellules,' when sufficiently young, that, owing to the vitelline granulations which indicate their shapes by enveloping them on all sides, one often divines them rather than sees their contours." (p. 373.) I think it must be that they are "divined" in all cases where the examination is made before the formation of the polar globules. He says himself that they are much more easily shown before than after that event (p. 381).

He recognizes the existence of a spindle, but considers it of little importance. The equatorial enlargements of its fibres are not prominent, although sufficiently evident in most cases. They consist of a gradual thickening of the rays up to the equator, or of nodosities distributed along the rays at the middle third of the spindle. They never appear under the form of a *nuclear plate*, an organ, says Pérez, to which is attached a great importance, since it is considered as destined to give rise to the nuclei which are subsequently seen at the extremities of the spindle, and, when the latter has ceased to exist, in its

place. As stated above, these nuclei have, in his opinion, an entirely different origin. The signification of the enlargements remains to be discovered, but they do not appear to him to be in all cases essential. Concerning received opinions about the formation, division, and migration of the nuclear plates, and their conversion into nuclei, he says: "Cette interprétation n'est nullement conforme aux faits observés par moi chez l'Helix, et que je viens d'exposer. Sans nier d'une manière absolute cette marche des radiations [?] du fuseau vers les sommets de ce corps, bien que je n'aie rien observé de semblable, je dois déclarer qu'il n'existe pas véritablement de plaque nucléaire chez l'Hélice. Existât-elle d'ailleurs chez cet animal, on ne saurait lui attribuer la production des noyaux du système radiaire, dont l'origine est toute autre, ainsi que je crois l'avoir démontré."

Pérez thinks Auerbach, Bütschli, and Strasburger are wrong in making the nuclei arise in the spindle, and not at the centres of the asters, and explains as the cause of their error that they have not witnessed the origin of these structures. When the nuclei are advanced in age, the dynamic influence which they exert on the surrounding protoplasm ceases, and they then move a little toward the equator. Manipulation and reagents may also cause or exaggerate this peculiarity. These, then, are the sources of error into which he thinks previous observers have fallen!

The mistakes of Pérez already pointed out, and his failure to discover the nuclear plate, are, I believe, chargeable with all this subversion of the real order of events, and to the same account must be attributed his misconception of the nature of the polar globules. I need not repeat the proof, which is entirely incontestable, of the cellular nature of these globules, and will only state briefly the position defended by the author. These globules are formed just as decribed by Robin, with the exception that the second is produced like the first, and is not, as Robin maintained, already formed before elimination. There is nothing in Pérez's description or figures of this stage which is not to be seen in the living egg. It is, then, not surprising that the globules are considered as only two drops of the disintegrated radial substance which once surrounded the "stellar nuclei," and that neither the spindle nor the half of it escapes as such from the yolk. It is not sufficient that he tells us he has "followed attentively the phenomena," and has "endeavored to discover that which these savants have described." There is no evidence that he has used *in these stages* the means necessary for the discovery of the things they have described, and it is therefore to no purpose that the assertion is made, "Leur structure n'a rien de l'élément cellulaire, et ils ne naissent point comme lui." He has "been able to recognize neither the spindle nor the two suns between the production of the first polar globule and that of the second." "It appears that with this mollusk there does not remain in the vitellus a single trace of radiate protoplasm after the emergence of the two polar globules." The figures which I have given for Limax show how probable it is that Pérez has overlooked some of the stages in the formation of polar globules in Helix.

In his opinion, the radiate substance surrounding the vesicular nuclei having

been eliminated as two polar globules, there are left in the yolk these two nuclei. They may, I think, unhesitatingly be considered the "pronuclei." He seems never to have observed them at any great distance apart, although "sometimes one sees them separated a little from each other, sometimes closely approximated." Each contains a single nucleolus. The nuclei grow. Their nucleoli undergo a series of irregular segmentations. These divisions do not seem to have been directly followed, but are inferred from the frequency with which one finds biscuit-shaped or constricted corpuscles. Pérez is unable to say what becomes of the two nuclei. He concludes there is no other alternative: either one of the nuclei disappears, the other alone being called upon to inherit the individuality of the ovule, and to assume the dignity of vitelline nucleus, or the two nuclei fuse. He has not seen the nuclei mutually approach, but has always found them at some distance from each other, never in immediate contact. For this reason, and on account of a theoretical consideration, — namely, that the amphiaster immediately preceding the first cleavage is the same as the first amphiaster, and must, like it and all later amphiasters, arise from a *single* nucleus, — Pérez is of the opinion that only one survives and furnishes a lineage, while the other perishes.

He has arrived at the conclusion that there is a genetic connection between the germinative dot and all subsequent generations of vitelline nuclei, but he has done so by the introduction of two fundamental errors; for the corpuscles at the centres of the asters are not derived, as he claims, from the germinative dot, nor do they, on the other hand, constitute the nuclei of succeeding generations.

The mistake concerning the origin of the two nuclear structures (pronuclei) to be found after the formation of the polar globules, deprives of its importance his negative statements relative to the penetration of spermatozoa. It may be, or it may not, that "fecundation is simply the result of the dissolution of the spermatozoön at the surface of the vitellus and of the absorption of its substance by the ovule." Reasoning from the analogy of cases easier of control and observation, it is highly probable that in Helix a penetration of the spermatazoön takes place. Nothing which Pérez has presented in the way of observation diminishes the probability that such is the case, nor that one of the nuclei he has seen is the equivalent of the male pronucleus.

Concerning the existence of a vitelline membrane, I think it is quite hazardous to speak dogmatically. It is rarely that I have seen in Limax evidence (Fig. 57, compare also Figs. 80^b, 80^c) of the existence of anything resembling such a membrane. That, however, does not prevent its constant occurrence in the case of Helix. I am led to suspect, however, by the statement that it is easily ruptured, that the author may have assumed that it previously existed in some cases where he had no direct evidence of its presence. At least, his Fig. 19 shows no trace of such a membrane.

Pérez has contributed very interesting observations on the nature of the egg just before the formation of the polar globules. No one, I believe, has seen such strongly marked and numerous pseudopodal protrusions of the vitellus of

any pulmonate mollusk as he has shown in Figs. 4 and 5. These irregularly conical, pointed projections of hyaline protoplasm may sometimes attain two fifths the length of the radius of the yolk, and the larger ones exhibit prolongations of the granular vitelline substance for a little distance into the central portion of their bases. They are normally radial in position, but are easily distorted by manipulation of the egg. The only motion which he has recognized has been a slight displacement of the granules mentioned, doubtless caused, he thinks, by unobserved changes in the form of the cones. These persist for a comparatively long time ; but they are at length contracted into knoblike processes with narrow pedicels, and then entirely disappear. "Their presence coincides with that of the system of radiations which succeeds the germinative vesicle, and they are always retracted within the yolk before the escape of the first polar globule." They recall that which Fol has named the "cône d'exsudation" in the egg of Asterias. "But I am able to affirm that, with Helix, there never exists between these hyaline cones and spermatozoa the relations which this savant has attributed to them in the case of the echinoderms."

The pseudopodia which were seen in Limax (Fig. 95) were limited to the region of the animal pole, were much lower and more rounded, and occurred at a later stage, than those seen by Pérez in Helix.

FOL'S ('79) finely illustrated memoir on "Fecundation," etc., besides considering more fully than any of his preliminary papers the events of maturation and fecundation, contains extensive bibliographical abstracts and criticisms, a chapter on segmentation, and the discussion of several topics of theoretical interest.

The author's observations were made on representatives of *echinoderms* (Asterias, Sphærechinus, Toxopneustes), *worms* (Sagitta), and *mollusks* (Pterotrachea).

In Asterias the germinative vesicle, "nucléus de l'ovule," is composed of a liquid portion surrounded by a viscid, semi-fluid limiting layer, which does not merit the name of membrane, in the ordinary sense of the word. Hardened in alcohol or one of numerous acids, the liquid part of the nucleus of a mature ovarian ovule is surrounded by a membrane presenting a double contour. This does not warrant the conclusion that a membrane exists in the living egg, but only that the vesicle is limited by a layer which coagulates differently from the surrounding vitelline substance. The discussion as to whether this layer belongs to nucleus or vitellus is useless. It is only to be determined by tracing its origin. This is difficult in the germinative vesicle, but not in the female pronucleus. The latter is formed — both its contents and its limiting envelope — in the midst and at the expense of the vitellus. "Nucleus" embraces both the envelope and the contents. The fluid contents are traversed by a network of sarcodic filaments uniting two layers of like substance, one of which lines the envelope within, while the other surrounds the germinative dot. The filaments hold in suspension pale, sparse granules of variable magnitudes. With the use of reagents a greater number

of them are distinguishable. When the germinative vesicle is about to disappear, the network is no longer to be found, even after employing reagents. This is proof that it is not an artificial product. The germinative dot is highly refringent, embraces one or several vacuoles, but no granulations, and is not surrounded by any layer different from the rest of its substance.

It will be unnecessary to give the details of the metamorphosis of the germinative vesicle and dot, which have been briefly reviewed elsewhere (p. 436). I will only mention some points of particular interest.

With the flattening of the space (lacuna) which represents the disappearing vesicle, the substance of the dot changes form, and becomes pale. In some cases the granular substance of the yolk was seen to encroach upon the "lacuna," especially on the side nearest the surface of the ovule, and to unite with the remnant of the germinative spot; in other cases the remnant approached the periphery of the lacuna, but without penetrating into the vitellus, and sometimes its position appeared to follow no law. The results from hardened eggs were conflicting as to the connection of the nucleolus with the forming amphiaster. In some cases the bipolar rays, emerging from an aster lying outside the lacuna, abut upon a cluster of granules which are the disintegrated germinative spot; occasionally a remnant of the spot is joined by a refringent filament to one of the asters of an amphiaster; more frequently the fragments of the nucleolus are widely scattered, and then one is certain that there is only a single aster, though subsequently the amphiaster is seen occupying a nearly horizontal position, and those few fragments of the nucleolus which remain visible are distant from it. The former cases would indicate that a minimum portion of the nucleolus contributes to form the amphiaster; the last, that they have no connection. The author "therefore prefers to consider the participation of the germinative spot in the formation of the 'amphiaster de rebut' as improbable, but without venturing to deny it absolutely."

Respecting the metamorphosis of the membrane of the germinative vesicle, he is unable to assert that it is entire, because of the folds which appear when treated with acids; but whether entire or not, it always forms the limit between the clear substance and the granular vitelline substance; it does not expel its contents, as Van Beneden erroneously thought. Whether the equatorial zone of fibre thickenings in the first spindle arises from the nucleolus or from the membrane, is left undecided.

The author still holds to the probability that the amphiaster first formed is not the "amphiaster de rebut." This opinion seems to rest on the oblique or horizontal position of the amphiaster seen in the earlier stages, as compared with the radial position observed later, and especially on certain preparations made with osmic acid, in which an oval corpuscle, embracing vacuoles and having a denticulate border, occupies the place of the horizontal amphiaster. This he thinks probably indicates a period of inactivity, during which the amphiaster, without ceasing to exist, masses itself together. I am not certain how this is to be harmonized with the subsequent statement that this corpuscle is probably only an amphiaster little accentuated and disfigured by the osmic acid. *Both*

explanations are not necessary. It appears to me that the one last stated is the more probable, and that consequently there is no ground for doubting the identity of the first amphiaster formed and that from the external half of which the first polar globule arises.

The eggs of Asterias are in some particulars less favorable for showing the manner in which the polar globule is formed, than are those of Limax. Nevertheless the results correspond very closely. From the greater size of the aster in Limax it has been possible to note more exactly the changes in the course of its rays as the amphiaster approaches the surface of the vitellus. Similar conditions doubtless prevail in Asterias ; for Fol says the external aster (when the protuberance appears) is only a half-aster, since its centre touches the summit of the protuberance. (Compare his Pl. II. Fig. 10.) The internal half of the amphiaster, says Fol, alone remains in the yolk ; the external half constitutes the protuberance, in which one still often sees remnants of the bipolar rays. At other times the external half of the amphiaster (spindle) promptly disappears, and is resolved into irregular corpuscles. These remnants are, to judge from the figures, the fibre thickenings of the external zone, which in the one case remain more regularly arranged; in the other, less so. The internal half of the aster, continues the author, preserves its structure intact, and an elongated enlargement is seen upon each of the bipolar filaments.

The detachment of this protuberance to form the polar globule, he thinks, differs in several important pacticulars from the corresponding changes in segmentation. Some of these differences are the same as those to which I have already called attention in the case of Limax ; others I believe to be of less general importance. " Nous ne voyons pas le globule s'arrondir au point de ne toucher le vitellus que par une surface extrêmement petite, et s'affaisser, une fois la division opérée, comme c'est le cas dans le fractionnement ordinaire. Le globule reste accolé au vitellus par une surface relativement large et la séparation n'a lieu que très-lentement, par un processus presque impossible à observer directement." This appears to me principally dependent on the presence of the " Oolème pellucide," which envelops the ovum. It is rarely that anything of a similar nature is observable in Limax (Fig. 57), where there is nothing to interfere with the free course of the division. In the second place, says Fol, the rays of the amphiaster (spindle) cut in two do not immediately withdraw toward the centres of their respective asters to contribute to the formation of the nuclei of two new cells. Those which belong to the polar globule persist a long time distinct, and the varicosities remain some time after the division is accomplished. Subsequently there are seen in the globule granules and vacuoles, irregular both in form and arrangement. A long time after the formation of the globule these parts are so arranged as to form a relatively large nucleus surrounded with a layer of sarcode. It is unnecessary to dwell on the significance of these peculiarities ; they are the same as in Limax. The re-formation of the fibre thickenings into a nucleus does not occur with the same rapidity in all cases; but in Limax it may occur almost as promptly in the second polar globule (Fig. 60. *rn.*) as in the formation of the

female pronucleus. On another point our conclusions are less in agreement. Fol says the half of the amphiaster remaining in the vitellus contracts and passes through a short period of repose. Its arrangement remains the same as during the formation of the first polar globule, but it momentarily fades a little. It becomes more distinct when the work of expulsion recommences; the bipolar filaments elongate, the aster becomes larger, more marked, and farther removed from the surface. The elongated bipolar filaments form again a fusiform body, the interior extremity of which corresponds to the centre of the aster, while the exterior extremity is found at the surface of the vitellus. This external point of convergence is not yet surrounded with rays; but unipolar rays soon appear around it, so that exactly the same figure is produced as at the formation of the first polar globule. The exterior aster of the second amphiaster almost always lies at one side of the point of contact of the vitellus with the first globule, and the axis of the amphiaster is usually oblique. His positive assertions that the internal half of the amphiaster is not massed into a nucleus, but is transformed directly into a new amphiaster, makes me somewhat distrustful of the conclusions I have expressed on this point; it is at least of some significance that the granulations at the internal ends of the spindles in my Figs. 22, 25, 40, are less conspicuous than the corresponding thickenings in the polar globules. I, however, still believe this feature deserving of renewed investigation. Fol believes the peculiar position of the centre of the external aster at the apex of the elevation is explained by the nature of its physiological *rôle* expressed in the name, *sphérules de rebut.*

I can confirm for Limax (Fig. 80^c) what the author thinks probable in Asterias, namely, that the envelope of the polar globule is continuous with that of the vitellus, rather than that the latter is pierced by the forming globule, although I have not found the envelope especially thin at the summit of the polar cell (Figs. 22, 25, 40, 61–63).

During the constriction of the peduncle of the polar globule in Asterias, Fol has observed, as did O. Hertwig, the existence of folds in the vitelline envelope, radiating from the peduncle on all sides, and most prominent near its base. This he believes indicates the presence of a superficial layer more inert, less living than the protoplasm which it surrounds, but not the existence of a veritable membrane; a membrane, however elastic it may be, finally becomes detached from the yolk, and assumes its former position. This it does instantly under the influence of acids. But here the so-called membrane follows the division of the protoplasm, and forms an envelope around each of the segments, never resuming its former position, even if one employs acids before the division is effected. The same conclusions appear equally applicable to Limax, although in some cases (Fig. 80^c) it may be difficult to assert that the envelope has not reached a degree of differentiation which closely approaches that of a genuine membrane. I have only once seen anything like radiating folds at the surface of the segments, and then (Fig. 68) they were confined to the polar globule.

Of the formation of the female pronucleus in Asterias, the author says that

the various rays of the aster which remains in the yolk after the elimination of the second polar globule are amassed and disappear as such ; but their reunited substance, especially that of the bipolar filaments, forms a small corpuscle, transparent and difficult to see in the living egg, but very apparent when treated with osmic acid and carmine. It is at first immovable, and increases gradually in volume ; afterwards it moves from the surface, at first slowly, and then more rapidly. At the side of this arise other pale spots, which, at first very small, grow rapidly, approach the first one, following its centripetal movement, and finally fuse with it. The impression conveyed by this description is that *only the first pale spot arises directly from the spindle fibres.* The others are not constant in their position. Treatment with acids causes these spots to appear like nuclei, and one distinguishes a very irregular enveloping layer ; the smaller the nucleus, the thicker the layer. Within these small nuclei are one or several nucleoli, which grow at the same time as the nucleus. They are surrounded with rays which increase rapidly in extent during the growth of the nuclei, but disappear when the pronucleus becomes stationary. If this account of the origin of the female pronucleus is accurate for Asterias, there must be an important difference between it and Limax, for in the latter I am very sure there are no accessory nuclear vacuoles which are entirely independent of the spindle fibres.

He says these changes transpire the same, whether the eggs have been fecundated or not, except in regard to the position of the polar globules (inside or outside the vitelline membrane) ; but *they occur a little more rapidly when the eggs have been fertilized.*

The maturation of the eggs of the sea-urchin offers little additional information. The amphiaster and the single polar globule are larger in proportion to the size of the egg than in Asterias, and once *two* rows of granules (zones of fibre thickenings) were seen, one near each pole of the globule.

The germinative spot is wanting in the very young as well as in the maturer eggs of a Sagitta, which the author names S. Gegenbauri. There are other species of this genus in which the nucleolus is present. The office filled by the nucleolus in the development of the ovule, the author therefore concludes, cannot be one of primary importance. The place of the large germinative vesicle, which has grown smaller and disappeared, is occupied, before the exclusion of the egg, by a compact corpuscle with stellate borders, in the interior of which is to be distinguished, after treatment with very weak solutions of osmic acid and bichromate of potash, a vertical row of small refringent grains, the optical section of the plane which the granules constitute. This phase resembles that one which in Asterias was the cause of the author's denying the identity of the first amphiaster and that from which the first polar globule arises. The two globules are produced successively, and in fecundated eggs are restrained by the vitelline membrane, being forced into a depression which they cause in the surface of the yolk, but in non-fecundated eggs they are detached, and are retained only by the mucilaginous envelope. Here also these changes all transpire more slowly in eggs that have not been fecundated.

As was to have been expected, the events of maturation in Heteropoda show a greater resemblance to those in Limax than either of the other groups.

The author withholds judgment as to whether the limiting layer of the ovule is in this instance a true membrane, since he has not satisfied himself experimentally of its physical and chemical properties. In young ovules it has the aspect of a membrane, but its internal contour becomes less distinct in those that are mature. Whether it is resorbed or mingled anew with the vitelline sarcode, it does not exist after the exclusion of the egg. The nucleus of the egg at the time of deposit is identical with the nucleus of the ovule (i. e. germinative vesicle). It then appears in the living egg as a clear spot at the centre of the yolk, which soon vanishes, and the central part of the vitellus then assumes a more homogeneous aspect, in which, however, a radial figure is discernible. In about half an hour there appears on one side of the yolk a clear space resting with a broad base at the surface, and continuing toward the centre in the form of a cone. It is composed of protoplasm without any "protolecith." As it increases in size, the lecithic globules, especially near the surface, take on a radial arrangement about the centre of the clear space. In an hour and a half the protuberance of the first polar globule appears, and within it one can distinguish the bipolar filaments and their enlargements without the use of reagents. Two hours and three quarters after exclusion the first globule is entirely detached, and the radial arrangement of the "lecith" indicates the formation of the second amphiaster; at this moment there appears a voluminous protuberance at the nutritive pole, composed of protolecith and sarcode. The superficial layer of the latter is here thicker than over the other parts of the yolk. At the end of three and a half hours the second globule is fully detached, and the vitelline protuberance has meantime entirely disappeared. Prolongations often seen arising from the surface of this protuberance (Pl. VIII. Fig. 9) are trabeculæ resulting from the retraction of the albumen of the coagulated egg, and therefore do not pertain to the vitellus.* Fol is unable to give any explanation of the meaning of this protuberance. — The metamorphosis of the germinative vesicle as shown by hardened eggs confirms in many ways the views at which I had arrived. The vesicle at the time of exclusion is still quite distinct, provided with a limiting layer, and embraces a network of sarcodic filaments, but contains only a few irregular refringent granules in place of a nucleolus. The enveloping layer, the so-called membrane of the vesicle, becomes less distinct, although it still remains visible. The vesicle diminishes a little in volume, but preserves an almost spherical form, without shrivelling. At the opposite poles of this great rounded cavity one now distinguishes two masses of granular substance,

* It should be remarked in this connection, however, that the author subsequently (page 112) alludes to this as a protuberance "with its accumulation of protoplasm and sometimes *pseudopods at its surface* (Pl. VIII. Fig. 9, *Ev'*)." Since this is the same figure as that cited in connection with the description given above, it would appear that the author may have changed his opinion concerning these pointed elevations between the times of the two writings.

in texture exactly like that which surrounds the vesicle and stretches out between the globules of "protolecith." "*These masses protrude slightly into the cavity of the germinative vesicle, which otherwise remains perfectly rounded.*" * This internal limitation is therefore very easy to distinguish, but externally they are absolutely indistinguishable from the vitelline sarcode of which they form a part. From these masses striæ soon arise which take the direction of meridional lines. These become more distinct, and are changed into [?] veritable filaments. Falling short of the equatorial plane, they do not yet encounter each other. During all the phases of their formation, *the peripheral extremities of these filaments are in continuity with the protoplasmic network which occupies the interior of the nucleus.* As the rays advance, the network disappears. It is more than probable that the rays are only a modification of the form of the intranuclear network, and that they result from a regular arrangement of its trabeculæ. This view of the origin of the spindle fibres is not directly reconcilable with the one I have expressed; nevertheless, I see no occasion to modify the argument based on the great distance which in Limax intervenes between the nucleus and the centres of the asters. The account of the origin of the polar masses I will give in the words of the author.

"Quant aux amas polaires, leur origine première est bien plus difficile à établir. J'avoue que, pour ma part, je n'y suis pas parvenu et qu'à cet égard je ne puis que poser une alternative sans la résoudre. Ces amas peuvent provenir du sarcode intranucléaire qui se porterait aux deux pôles opposés du noyau et se confondrait avec le protoplasme vitellin, ou bien ils peuvent provenir du protoplasme périnucléaire qui ferait irruption dans la cavité de la vésicule; à moins encore que ces deux processus ne se produisent simultanément, et qu'il n'y ait, dès le premier instant, une fusion entre ces deux substances. Que cette fusion soit immédiate ou non, il est incontestable que les protoplasmes intra- et périnucléaire ne tardent pas à se confondre aux deux pôles, en sorte que, un peu plus tôt, un peu plus tard, il y a toujours fusion.

"Les amas polaires faisaient d'abord une légère saillie dans l'intérieur de la vésicule sphérique. Pendant la croissance des rayons intranucléaires, ils s'éloignent du centre et font de part et d'autre hernie dans le vitellus. Il en résulte que la vésicule passe de la forme sphérique à celle d'un citron très-court. Pendant ce temps les rayons nucléaires, qui se trouvent près de l'axe qui rejoint les deux pôles, sont arrivés à se rencontrer et se sont soudés de manière à constituer quelques filaments bipolaires; les rayons latéraux de chaque aster vont encore se perdre dans le réseau intranucléaire."

The extranuclear rays arise at the same time as the intranuclear, and the growth of both is exactly alike. There is therefore a time during which each centre of attraction is surrounded by a system of rays without being yet joined to that of the neighboring aster. The amphiaster occupies at first an eccentric position. The small grains representing the nucleolus may possibly go directly to the spindle, since granules of entirely similar appearance are seen along the intranuclear rays when the amphiaster is still incomplete. The

* Not italicized in the original.

author, however, doubts the genetic connection, since these granules are often entirely wanting. Subsequently the amphiaster is completed by the welding of the intranuclear rays end to end, and the "granules de Bütschli" make their appearance as enlargements of the bipolar filaments. But the relation of these enlargements to the grains presented by the still isolated rays remained obscure. The amphiaster elongates, and at the same time stretches the membrane of the vesicle. The vitelline rays have increased in extent, and the centre of each aster is occupied by a few granulations, around which is a space occupied by homogeneous protoplasm. Meanwhile the membrane of the germinative vesicle assumes indefinite contours and entirely disappears. The amphiaster moves toward the periphery; at first oblique, it becomes perpendicular to the surface, with which the centre of one of the asters becomes almost "flush." Then the surface is raised into a dome, and the enlargements of the bipolar rays divide; the first polar globule, composed of half the "amphiaster de rebut," is detached. The internal half undergoes the same modifications as in Asterias, but the second amphiaster is smaller than the first. Portions of the bipolar filaments and their enlargements are readily distinguished at, and some time after, the formation of the globule. The enlargements all lie at the same height; at the time of segmentation the polar globule has assumed the appearance of a cell with a large nucleus, and one or several nucleoli. They decompose, and have no part in the development of the egg. The views of the author, it will be observed, seem to have been modified in some particulars since the publication of his earlier paper on Heteropoda. See pp. 429, 430.

The principal events of fecundation as described for Asterias have already (pp. 480, 486) been given. It is necessary to add only a few particulars. The "cone of attraction" may extend to half the thickness of the mucilaginous layer if the spermatozoön advances slowly, but is much shorter and more rounded when it approaches quickly, for as soon as the contact between the two is effected, the cone commences to retract. Most spermatozoa enter the nutritive hemisphere, but one often sees a penetration in the formative half, even up to the immediate vicinity of the polar globules. At the moment when a space appears under the vitelline membrane around the point of fecundation, the differentiation, but not the elevation, of the membrane has extended quite around the vitellus. From this instant the egg is inaccessible to every spermatozoön which reaches the membrane; for the vitellus is no longer able to produce a "cône d'attraction," and in Asterias a spermatozoön is hardly capable of penetrating without the aid of this excrescence. The space embraced between the elevated membrane and the yolk is occupied by a transparent substance, which cannot be a liquid, but must be a very clear jelly, since, if it were a liquid, the vitellus would change position, and the space could not remain of uniform thickness all around. Does this substance arise exclusively as a secretion from the yolk, or is there at the same time an imbibition through the vitelline membrane? If the former, the vitellus should suffer a diminution of volume. It is difficult to determine whether this is so, on account of the

changes in the form of the yolk. If the latter diminishes in volume, it can be but little. The vitellus and membrane have a greater diameter than existed before the formation of the membrane. The author therefore speaks of an elevation of the latter, and not of a retraction, which appears to him doubtful. The orifice through the membrane at the "crater" which gave exit to the "cone of attraction," and possibly existed during the early stages of the formation of the "cone of exudation," is no longer to be found after the complete dispersion of the latter cone, nor is the crater longer visible. Directly underneath this "crater" of the membrane there is a corresponding but smaller depression in the surface of the yolk. This is still visible when the membrane is wholly elevated, but before the male pronucleus is formed. The latter appears as a small clear spot without granules immediately under the vitelline crater.

The phenomena occurring in the sea-urchin have been considered at page 490. The vitelline membrane is elevated with greater rapidity and energy than in the case of Asterias. The zoösperm suffers little change of form at penetration. It enters progressively by the action of the vitelline sarcode, and is not impelled by its cue, which has ceased its undulatory movements. The "cone of exudation" is extremely pale and very mobile. The author does not know whether this is a phenomenon of amœboid contractions or a continuous eruption of an almost liquid substance. The body of the spermatozoön once plunged into the yolk is often visible without the aid of reagents. The point of penetration is only determinable by the fact that the female pronucleus retires only part way from the formative pole toward the centre of the yolk. With this as a criterion it may be shown that the penetration takes place at any point, but perhaps more often in the nutritive hemisphere.

The growth and union of the pronuclei is nearly the same in starfish and sea-urchin. In Asterias the clear spot where the zoösperm penetrated becomes the point of departure for the male pronucleus, which at first remains for several minutes immovable and without apparent change. The vitelline rays are all directed toward the centre of the spot; some of them are slightly curved so as to abut at the point of the surface where the cone of exudation still persists. The rays become longer and more accentuated with the advance of the aster into the yolk. Its direction, at first centripetal, changes when the female pronucleus does not occupy the centre of the egg, so as to encounter the latter. If the egg is fecundated before the completion of the polar globules, the male pronucleus remains at the edge of the yolk in the condition of a small, hardly visible spot until they are eliminated. Both pronuclei then arise simultaneously. In this case they meet between the centre and the formative pole, because the male pronucleus advances more rapidly.

In the sea-urchin, while the clear spot is contiguous to the surface, its interior often shows a rounded refringent globule, which appears to correspond to the body of the spermatozoön modified in form, and soon becomes in the living egg invisible. Treatment with osmic acid and carmine shows that the zoösperm preserves a few instants its conical form, then becomes rounded into

a strongly colored corpuscle, which is surrounded by a clear area and rays. In approaching the female pronucleus, the corpuscle increases to nearly double its original size. Preparations made when the female pronucleus is already surrounded by rays of the male aster show that the nucleus is almost constantly oval, and drawn to a point on the side nearest the male pronucleus. The nature of the male pronucleus is especially elucidated in the star-fish. It is sometimes only as large as that of the sea-urchin, but at other times twice as large; in the latter case it no longer has a homogeneous appearance, but is surrounded by an enveloping layer which is darker than the contents. The cause of this difference is unknown, but it establishes a transition between the condition shown by the sea-urchin and that of the Heteropoda where the two pronuclei have the same size and texture. Were it not for this transition, it would be difficult to ascertain whether the dark corpuscle of the sea-urchin corresponds to the pronucleus of the Heteropoda or only to its nucleolus.

Besides the results already (p. 479) mentioned, the study of the pronuclei in Sagitta have afforded other points of particular interest. The altered form, which I ventured to assume for the male pronucleus, is actually encountered, and corresponds almost exactly with that of the pronuclei in Limax, Fig. 68. I extract the following from the author's description of the changes. Although fecundated at the moment of deposit, the vitellus shows a male aster only at the time when the polar globules are formed. It probably exists already at the edge of the yolk, but it must be quite small, since it escapes observation. Soon after the elimination of the globules there appears near the surface of the yolk, usually at the nutritive pole, a round or oval vacuole, the male pronucleus. The female pronucleus appears almost at the same instant. They move toward the centre, increasing in size, and meet between it and the formative pole. The female pronucleus is without an aster, that of the male grows rapidly, and lies in advance of the pronucleus. *The cavity of the male vacuole is surrounded by a sharp margin, except at the place where it touches the centre of the aster. There it appears open, as though the contents of the cavity passed by gradations into the substance forming the central mass of the aster. The vacuole always assumes the form of a melon-seed.* This description corresponds in almost every particular with the condition in Limax alluded to above. The author, believing from the appearance that the pronucleus is drawn on in a passive condition, and that the agency must be sought in the male aster, endeavored to show by reagents the presence of the body of a zoösperm, or a compact corpuscle, in the centre of the aster. Failing in this, "he must consider the vacuole and the central mass of the aster taken together as the homologue of the male pronucleus of other animals." This conclusion, if extended to the cited case in Limax, would involve one in the necessity of identifying the central area of the same aster with both male and female pronucleus; and in Sagitta certain stages in the approximation of the pronuclei (*op. cit.*, Taf. X. Fig. 7) appear to present the same difficulty, for the relation of the female pronucleus to the aster is at this stage essentially the same as that of the male

pronucleus. In Sagitta both are "open" on the side toward the centre of the aster, and in Limax both are drawn out in the same manner, and their outlines become less conspicuous on the side toward tne aster. But while Fol represents the line which indicates the contour of the pronucleus in Sagitta as terminating rather abruptly, I have simply seen the outline become very gradually less distinct, but never wholly interrupted. The pronuclei in Limax present the same smooth, even contour on the side toward the aster as elsewhere ; it is only less conspicuous, not less precise, on that side.* There is, besides, this difference in the two cases : in Sagitta the aster *arises* in connection with the male pronucleus, but in Limax in connection with the female.

At this stage (Pl. X. Fig. 7), continues the author, a corpuscle is generally seen suspended in the liquid of the cavity of each of the vacuoles, near the side with which they are about to come in contact. They are very distinct, owing to the low refractive power of the liquid, and are comparable to the nucleoli found in the pronuclei of other animals. The pronuclei have the form of a grape from which the stem has been torn; it is by this truncate side that they approach each other, separated by only a thin layer of vitelline substance. Some of the rays of the aster now converge toward the space which separates the two pronuclei, and the others toward the inferior † extremity of the male pronucleus. When the pronuclei meet, the rays extend around both, converging toward the line which separates them. In coupling, they are mutually flattened. Fol's Fig. 10 seems to indicate that they are no longer "open" when this flattening begins. They always deport themselves optically, he continues, like vacuoles full of liquid in the midst of a denser substance. The contours are perfectly distinct, but simple and without indication of a membrane or limiting layer. Variable sarcodic masses are visible within the pronuclei. The rounded mass (nucleolus) of the preceding stage has disappeared, and in its place are seen sometimes filaments, sometimes partitions, at other times streaks of sarcode stretching across the cavity in various directions, and exhibiting enlargements of all forms and sizes. The stars of the first cleavage amphiaster evidently arise in Sagitta, also, before the fusion of the pronuclei ; for the author says that, when considerably flattened, there often appear at their opposite lateral edges small lenticular masses which project into their cavity. (Compare *loc. cit.*, Taf. X. Fig. 9.)

The corresponding events in the Heteropoda offer many points of resemblance with Limax. In one place the author speaks incidentally of the multiple condition of the female pronucleus. When it is composed of two or three small nuclei, each of them, he says, contains its own nucleolus. The figure

* From the difficulty of rendering a sharp outline on stone with the crayon, the pointed ends of the pronuclei in Fig. 68 are not quite so definite, especially in the later prints, as they should be.

† From the figures cited it is evident that the blunt end of the pronucleus is meant, although it is *uppermost* in the figure. The description may date from a period before the author began to deviate from the customary method, by placing the vegetative pole of his figures uppermost.

cited (Pl. I. Fig. 13) is the only one which shows such a condition, and even in this one of the small nuclei is so covered by the other that the proof of their independence is not conveyed by the figure alone. If this condition really is met with, it must be very rare, for the author would otherwise have given a more detailed account of it. The internal star of the second amphiaster is much less developed and disappears earlier than in Limax. At its first appearance the male pronucleus is situated just underneath the surface of the yolk, rarely in the immediate vicinity of the nutritive pole, though more often in the nutritive hemisphere. It has no relation with the protuberance at the nutritive pole. He believes the latter is more accentuated when this pronucleus arises in the formative hemisphere. Both pronuclei develop with the same rapidity and in the same manner; each soon presents a large nucleolus in its interior; but the female pronucleus advances little or not at all toward the centre of the vitellus, because [?] it is soon joined by the male pronucleus, whose motion is infinitely more rapid. After treatment with picric or acetic acid the pronuclei are, at the moment of their appearance, homogeneous. A little later a certain number of small spherical grains, each of which is furnished with a black point in its centre, appear in the interior. Still later the pronuclei present the vesicular character of true nuclei, the limit being formed by an irregular layer of variable thickness. Neither osmic acid nor alcohol causes this layer to appear. The contents remain clear and transparent after treatment with osmic acid, but become granular with the other reagents mentioned. The nucleolus is variable in different eggs. More often there is only a large one in each nucleus, but it often happens that there are instead several small nucleoli. Since the latter condition occurs in less advanced stages, it may be that the nucleoli become fused, or that one is developed to the exclusion of the others. The rays which surround the male pronucleus during its displacement, and are visible in the living egg, disappear after the use of reagents. It is possible that the same may be the case with Limax. Fol has given no figures of this stellate arrangement, but his statement is explicit. The two pronuclei may have attained their full size at the time of contact, or "they may be still relatively little developed (Fig. 7), and in the latter case the conjugated nucleus will be obliged to increase after its formation." I doubt if this last statement is warranted by the figure cited. There does not appear to be here, more than with Limax, a veritable conjugation nucleus. Unless I misinterpret this figure, it shows already the beginnings of the amphiaster of segmentation, and there cannot well be a further growth, but only a metamorphosis of the two pronuclei into a segmentation spindle. The nucleoli, continues Fol, still exist when the pronuclei are in juxtaposition, but they disappear at the moment when the latter are fused. During this fusion picric acid still causes the enveloping layer to appear, and, within, granulations arranged in lines diverging from the point of union.

The results of the fecundation of immature or over-ripe eggs of Asterias, or such as are taken from animals kept in confinement, all being abnormal, have been given at pp. 484, 485, 491.

The phenomena of segmentation were most extensively pursued in Toxo-

pneustes. After fecundation the vitellus remains in repose for about twenty minutes. There is a collection of transparent substance which forms an irregular layer around the central nucleus. The radiations in the yolk appear before the nucleus has suffered reduction of volume; they are optically like the substance which surrounds the nucleus, not like that of the nucleus itself. They diminish in breadth at the moment the latter is converted into an amphiaster. Auerbach's theory is refuted by these facts. Subsequently the nucleus is a little elongated, and the perinuclear protoplasm takes the form of a disk surrounding the nucleus, as the ring of Saturn does its planet. The disk is oval; when seen in profile the vitelline rays appear to diverge from it like the barbs of a feather. Treated with acetic or picric acid, the radial structure, contrary to the effect produced in subsequent stages, becomes *less* distinct. This phase lasts about twenty minutes. The protoplasmic disk meantime gradually diminishes in breadth and increases in length. Then it promptly becomes limited to two masses quite distinct from each other. The rays are no longer arranged like barbs, but like the spokes of a wheel. The nucleus becomes indistinct; reagents cause it to reappear in the form of a lemon. At the pointed extremities the limiting layer of the nucleus projects outward, and serves as centres for the two systems of rays. In acetic acid the latter are seen with great distinctness; they are without enlargements, and are soon lost in the midst of vitelline substance of uniform appearance. One or two of the granules in the nucleus are distinguished by their size and greater refringency, — perhaps a nucleolus in process of dissolution. Osmic acid confirms the existence of these conditions.

Transitions from this to the next described phase are not often met with in the sea-urchin, but are more readily found in the heteropods, in connection with which they are described.

In this next phase the spindle with equatorial fibre thickenings is already formed. It is during this stage that the second vitelline membrane begins to be detached. After treatment with picric acid there is no trace of an enveloping layer (membrane) around the nucleus; each aster is composed of distinct parts: a central, nearly spherical, clear, protoplasmic mass; a peripheral granular part, dark especially in the vicinity of the central mass, and of a radial texture remarkable for its delicacy and regularity. The dark substance terminates abruptly with a regular contour, but is not separated from the central mass by any membrane or envelope whatever. The limits are less pronounced, but not wanting, on the side toward the old nucleus. The centre of the clear portion is occupied by a cluster of granules, toward which all the filaments are directed. They stop at the edge of the clear mass; it is exceptional to see a few of the *intranuclear* filaments send pale prolongations as far as these granules. Rotation of the egg shows that the spindle is flattened so that its cross-section is elliptical, and that the cluster of granules at the centre of the aster has the form of a crescent, and therefore appears in section as a round body of limited extent. Treated with osmic acid, the extranuclear rays are almost obliterated, so that the central mass appears with greater distinctness. In acetic acid the unipolar rays are seen with surprising clearness, and a remnant of the nuclear

envelope becomes visible. This pseudo-membrane surrounds only the middle part of the nucleus ; it is wanting at the ends. The grain at the middle of each filament is evidently a simple enlargement of its substance. The unipolar rays are extremely delicate toward their extremities, and at one point are much swollen. Unlike those of the interior of the nucleus, — which are more rounded, distinct, and refringent, and perfectly regular in their arrangement, — these enlargements are elongated, variable in form, and placed at irregular distances from the centre of the aster, so that the enlargements of consecutive filaments never lie adjacent to each other. There may be filaments with two enlargements, others with none. The effect of staining in gold chloride appears to be, to a certain extent, an indication that the view I have expressed about the nature of the spindle fibres at their initiation is erroneous. Fol observes with this treatment that the asters assume a beautiful dark violet color, which at the periphery gradually merges into the color of the yolk, which is of a rose tint. The nucleus and the intranuclear rays, without being destroyed, remain pale, — "are not more stained than the rest of the vitellus."

The next phase is characterized by the division of the fibre thickenings, which cannot be observed, however, in the living egg. The vitellus changes form, now in one direction, now in another, but ultimately elongates in the direction of the axis of the amphiaster. In picric acid the enlargements, after division, appear larger and more elongated than in the preceding stage. The interzonal filaments are very pale, and soon disappear ; they are named "filaments connectifs." The flattening of the amphiaster increases, so that the spindle and areas appear in one position twice as broad as they do after being rotated 90° about the axis of the spindle. The granules of the "area" (sarcodic mass) are also extended in the same plane in the form of a cylindrical bolster, which may be straight or slightly curved. The extranuclear rays form a compact zone around the "area," and *appear* composed of pieces in juxtaposition like the bricks in an arch. Toward the exterior these pieces are continuous with granular rays. This structure is of limited extent, and since it exists at precisely the time when the rays in the living egg extend to the periphery of the yolk, it is to be concluded that the rays consist of two distinct parts, of which one (the central) is brought out by picric acid, while the other (peripheral) is only seen in living eggs. The author cites two figures (Pl. VII. Figs. 9, 11) to illustrate the condition shown at this stage after treatment with acetic acid. The description relates principally to Fig. 11. The nuclear filaments are not distinguishable from those of the vitellus, and the granular mass at the centre of the aster, not being discernible, is probably veiled by them ; but the region which extends between the two groups of intranuclear enlargements * is not thus covered ; one should therefore be able to see the filaments which connect these enlargements in pairs,† if they exist. It is easy,

* In Fig. 11 no such groups are represented, unless, as the author may possibly have assumed, they are already fused with the "sarcodic mass" at the astral centres.

† Fig. 9 shows a spindle with *equatorial* enlargements ; consequently the "connective filaments" are not to be sought in that stage.

on the contrary, to be assured of the absence of every connective filament in this region; it is occupied by a uniformly granular vitellus. Since acetic acid has the effect of making all sarcodic filaments so distinct, this fact appears to the author significant [of what ?]. I think no difficulty can be experienced in interpreting Fig. 9; it is Fig. 11 which still remains to be explained. I believe it corresponds to my Fig. 82. That the latter does not exhibit a stage *subsequent* to the formation of the equatorial plate is evident from a comparison with Figs. 90–93. I have assumed that it corresponds to a stage preceding the formation of a veritable spindle. The principal difficulty with this, as with Fol's interpretation, is in explaining what has become of the substance which usually appears at this time in the form of spindle-fibre thickenings. To assume, as Fol does, that this substance has already passed through the stages of division and migration, is in contradiction with every other figure he has given. I am not sure that both these cases may not represent abnormal conditions, — either a more complete dissolution and distribution of the substance of the nucleus than is usual, or a failure of the vitellus to respond as promptly as usual to the changes in the nucleus. It is perhaps possible that Fol's Fig. 11 represents a stage nearly corresponding with that of his Fig. 14 (Pl. VII.), and that the envelope of the nucleus has simply disappeared a little sooner than usual. In that event, there might be some reason, even in his observations, for retaining the view that the spindle fibres are at first composed of vitelline filaments. But however that may be, further observations are necessary to render either of these figures (Fig. 11 or Fig. 82) satisfactorily intelligible.

During the next stage the constriction of the yolk begins, and the second membrane is detached on all sides, although it follows the constriction for a certain distance. The "grains de Bütschli" reach the sarcodic mass of their respective asters, at the edge of which they appear as small spherical bodies, sometimes still arranged in a plane parallel to the equator, sometimes without order. They vary in size and are hollow. At the opposite margin of each "area" is another group of much smaller globules. The latter are still abundant, and derived in all probability from the central granular masses of the asters. Ultimately these two groups are intermingled. This may take place before the larger globules have become hollow, or not till after they have in addition each acquired a nucleolus.

The stage which follows ends with the separation of the segmentation spheres. The asters continue to move apart and pass the centres of their respective spheres; the "areas" have become conical or pyriform. The rays are curved, as already described by Auerbach. The vitelline membrane has failed to follow the furrow, and stretches across it from one sphere to the other. In picric acid the larger globules of the "area" are increased in size, and each contains a nucleolus. They are, therefore, true nuclei. The larger they (nuclei) are, the less their number, from which it is probable that they unite with each other. Their arrangement is irregular. The rays on the outer side of the "area" converge toward a point at the external side of the mass; all the other rays, toward the centre of the mass. In osmic acid the exterior form of the yolk

and its membranes is better preserved than in picric acid. The vitellus as a whole still continues flattened, and the same peculiarity affects the "areas" and the contained globules. The bodies of the centre of the aster appear small and homogeneous. At first located in the centre, they subsequently approach the young nuclei, with which they ultimately unite. Certain pale corpuscles between the first and second membranes are not polar globules, but result from a precipitate formed in the albuminous fluid by the reagents. The descriptions and figures of the corpuscles which occupy the centre of the aster do not seem to me to afford satisfactory proof of the conclusion, that they are fused with the new nuclei. Figs. 13, 14, of Fol's Pl. VI. show thickenings in the interzonal filaments.

In describing the formation of the polar globules in the Heteropoda, Fol expresses his belief that all the "matières de rebut" eliminated from the egg correspond to a *single* cellular element. Here, too, the female pronucleus is formed by a fusion of the central corpuscle of the deep aster with the compact corpuscle formed at the expense of the "renflements de Bütschli." As he has seen only *one* such corpuscle result from the enlargements, he thinks there is every reason to believe that the supplementary small nuclei (which he finds here) are formed, as in Asterias, independently of the first pronucleus in the substance of the central mass of the internal aster.

The first segmentation in the Heteropoda is as follows. The pronuclei become mutually flattened, the enveloping layer disappears from the surface of contact. This region of contact is the centre of an irregular system of diverging rays extending inside as well as outside the nuclei. This might be mistaken, he says, for the origin of the amphiaster; but by comparisons he has convinced himself that these first radial striæ correspond only to the molecular activity which is developed at the moment of the fusion of the nuclei, and that it disappears before the amphiaster arises. However it may be with the Heteropoda, I believe it is not thus with Limax. According to the further description, the plane of union is still visible in Heteropoda after the stars of the amphiaster have appeared. The latter always fall at opposite margins of that plane. At other times the fusion is more complete when the asters arise. The pronuclei meanwhile migrate nearly to the centre of the yolk. The contours of the (conjugated) nucleus remain visible up to the moment when the intranuclear enlargements are grouped in the vicinity of the centre of each aster. The middle region of the "filaments connectifs" (Pl. IX. Figs. 8, 9, 10, *Ft*) is composed of fine fibrillæ, which, the author states, have been incorrectly engraved, so that they appear like thickenings of the filaments. The spaces around the centres of the asters are occupied by *granular* protoplasm exhibiting a radial structure. Perhaps they correspond, he says, to the sarcodic masses which occupy the same position in the sea-urchin. The vitelline filaments of the latter would then correspond to the radial trails of protoplasm which stretch out between the lecithic globules of Pterotrachea. In that event equivalents of the rays immediately around the centre of the aster in the mollusks would not exist in the sea-urchin, or would be invisible by reason of the homogeneous nature of the sarcodic mass.

Compared with the first "amphiaster de rebut," the present amphiaster is characterized by the absence of vitelline rays, so prominent in the former, and the presence of this granular mass of protoplasm, which is wanting in the other. The axis of this amphiaster is curved, with its concavity directed toward the formative pole. It may be that this curvature sustains some relation to a vitelline protuberance which is at this moment visible at the nutritive pole. The author is ignorant of the signification of this protuberance, as he was of that which arises at the formation of the polar globules.

In the following stage the groups of fibre thickenings have approached closely the central corpuscle of each aster. The protuberance of the nutritive pole begins to be separated from the vitellus by a circular constriction; otherwise the yolk is perfectly rounded, and shows no indication of a segmentation furrow.

This furrow makes its appearance in the next stage around the vitellus on all sides; it passes to one side of the protuberance. Bütschli's corpuscles unite, on both sides, into two or three nuclei, which at once become swollen, and assume the appearance of vesicles, each with an enveloping layer and embracing irregular granules. These vesicles, some or all of them, become elongated in the direction of the central corpuscle of the aster, and present an opening like the neck of a bottle, which is extended almost into contact with the central corpuscle. The vesicles fuse into a single one, having the same form, thick walls and a large corpuscle, which is drawn to a point on the side toward the areal mass. The latter has disappeared, without doubt by absorption into the nucleus, and the contents of the nucleus are in continuity with the clear substance at the centre of the aster, at the expense of which the nucleus seems to grow. While the segmentation is being accomplished and the new nuclei are growing and taking the place of the asters, the protuberance at the vitelline pole gradually disappears by fusing with that one of the spheres of which it formed a part; thus one of the products of segmentation is more voluminous than the other.

His researches on Sagitta are especially valuable, since made almost exclusively on *living* eggs. They confirm the results obtained from the study of the Heteropoda. *The first sign of the impending division is the formation of small masses of sarcode at the opposite extremities of the nucleus, which is still intact and spherical.* These small masses are optically like the vitelline sarcode, and cause a slight indentation into the cavity of the nucleus, which, though not prominent, is still readily appreciable on account of the perfect sphericity of the rest of the contour. The vitelline rays tend to arrange themselves about the extremities of the nucleus in place of converging towards its centre. Thus is quickly produced the dumb-bell stage. The central mass of the asters is perfectly homogeneous. The intranuclear trail differs from the surrounding vitellus only by the presence of the connective striæ (interzonal filaments), which are pale and poorly defined. The contents of the new nuclei are clearer and less refringent than their vicinage. The centre of the aster is often occupied by a dark corpuscle. In the interior of the nucleus pale, ill-defined streaks of

protoplasm are seen, which together resemble the tongue of a bell, and are joined with the central substance of the aster. During the second and subsequent segmentations these streaks of sarcode become at a certain moment much more distinct than during the first segmentation; they take special forms, which recall the stamens of a flower. There are from four to six of them, but their position is not constant. They attain their greatest distinctness only when the nuclei are so swollen as to be perfectly spherical. Although a morphological continuity of these trails (traînées) with the enlargements of the bipolar filaments appears improbable, it is not absolutely impossible. One might suppose that only a part of the enlargements serve to form the envelope of the young nuclei, and that another part persists under its primitive form [the trails] to become subsequently the intranuclear network. Whatever their origin, these trails of protoplasm disappear during the growth of the new nuclei, and contribute without doubt to the formation of the sarcodic network. The nucleoli make their appearance only a long time after the disappearance of these trails, so that they do not seem to have any direct relation with them.

The author arrives at the following conclusions concerning the process of segmentation in general.

The first precursory phenomenon is the appearance of a stellate figure, — a radial arrangement of the vitellus, of which the nucleus is the centre. At this moment the nucleus is still intact, but a little less distinct than before; this appears to indicate that there are movements, — forces which exert their influence at the same time upon the nucleus and upon the vitelline protoplasm. The refringency of the nucleus and the distinctness of its contours are the only things which are modified, up to the moment when the new centres of attraction appear at its opposite poles. The nature of these forces are far from being elucidated, but there are in all cases places where a gradual passage is established between the nuclear substance and the vitelline protoplasm; there are therefore points of fusion between the two substances. These centres persist a certain time under the form of corpuscles or of granular masses. The rays of the amphiaster appear at first in immediate contact with the centres, and then stretch out in all directions. They fall into two categories, according as they extend into the interior of the nucleus or into the vitellus. The former are the only ones that are joined end to end. Both kinds bear enlargements; but those of the extranuclear filaments have no other destination than to add their mass to that of the centre of the aster, while the intranuclear unite in the vicinity of the centre of each aster into one or a small number of corpuscles, which become swollen and unite into a single vesicle, and thus become the origin of the new nuclei. The corpuscles occupying the centre of the star also contribute to the formation of the nuclear elements, which continue to grow at the expense of the sarcodic masses of the asters. The "filaments connectifs" remain outside the new nuclei, and do not contribute to their formation. The new nuclei therefore absorb only a part of the substance of the old nucleus, and in return are united with substances which formerly constituted a part of the vitellus. The formation of the polar globules takes place by the

process of cell division. But the second "amphiaster de rebut" arises directly from the internal half of the first. One may compare the two polar globules to a cell originally single.

In the cases of superfecundation the union of two male asters to a female pronucleus results in a conjugate nucleus, which soon gives place to a tetraster, in which four equidistant stars occupy the corners of an imaginary square, the sides of which are formed by the intranuclear filaments. In division each of the four groups of filaments (spindles) shows a series of enlargements which divide and migrate as usual. The two groups migrating toward each corner of the square unite to form a single nucleus, so that from the eight groups there result only four nuclei, one to each aster. There are many variations from this more typical condition. Instead of a tetraster there may be a pair of parallel amphiasters, and according as the corresponding asters of these amphiasters are more or less intimately joined by their sarcodic rays, the condition of the tetraster will be more or less closely approached. This is followed by a corresponding segmentation into four spheres, which resemble in position the condition after the *second* normal cleavage. So at subsequent stages there are eight instead of four spherules, etc. In the planula stage the larvæ are irregular. The formation of a tetraster, and the division into four spheres at once, the author thinks, is not simply an abbreviation of events ; it is a more fundamental alteration of the normal process.

Where there remain male pronuclei which have not become fused with the female, (in cases where several spermatozoa have penetrated the yolk,) sooner or later each of these male pronuclei is resolved into an amphiaster, from which two nuclei arise. When two or three of the male pronuclei have united with a corresponding number of the components of the female pronucleus, and there are others which remain distinct, the conjugated nuclei give rise each to a tetraster, and the superfluous male pronuclei also divide, but with less regularity. All eggs embracing independent male asters are very irregular in their segmentation. All those which have received more than one spermatozoön give rise to at least twice as many segmentation spheres as would exist in a normal embryo of the same stage of development, and become monsters. This monstrosity consists in the repetition of a primitive organ which should normally remain single. Other results have already been given at pages 484 and 491.

Fol, rejecting the term "deutoplasm," introduced by Ed. van Beneden, proposes to distinguish the nutritive substances accumulated in the vitellus from those often deposited by the embryo in its interior, and to designate the former as "*protolécithe*," the latter as "*deutolécithe*."

He proposes further to preserve the term *membrane* only for the thin layers with double contour, which are harder and more resistant than the protoplasm, and which have lost the ability of remingling themselves directly as living substance with the living protoplasm. He would class under the name of limiting or plastic layers (*couches limitantes* or *plastiques*) those which have the peculiarity of following the sarcode in all its changes of form, even the most extreme, and of re-entering directly into the protoplasmic circulation, together

with those which the protoplasm can easily and instantaneously traverse without being first obliged to dissolve them. Limiting layers, which have only a single clear contour, while the other surface passes by insensible transitions into the neighboring substance, may be given the name of "*pellicule.*"

Fol has also been impressed by the fact, that in the formation of the *polar globules* the centre of the external aster reaches the surface of the vitellus, and subsequently continues to occupy the most external portion of the globule until the latter is almost detached. Without having observed the curvature of the rays, he remarks that the aster is of necessity incomplete, and thinks these peculiarities should correspond to a difference (from ordinary cell division) in the mechanism and forces of division. The amphiaster is in some way expelled, pushed by a *vis a tergo*, instead of operating as two "centres d'appel." Since they subserve no function for vitellus or embryo, and soon suffer disintegration, he prefers the term globules or spherules to that of cells. Granting that there are objections to the use of "globules excrétés," he claims the justice of the name "corpuscules de rebut." They are small masses of a substance that has become superfluous, or rather injurious, to the egg, and are for that reason expelled. It is of little significance that this substance has, as germinative vesicle and dot, played an important part in the growth of the ovule, or that its mode of expulsion resembles the division of cells; they are none the less worn-out materials, and their constancy in the animal kingdom simply tends to show that these substances have become injurious, — an obstacle to "la fécondation intime" and to embryonic development. From all observations it appears that the expulsion of a part of the nucleus of the ovule may be a condition indispensable to the fusion of the pronuclei. If that is the case, one is naturally led to inquire if there are not in the germinative vesicle substances of different affinities or polarities. The combination of these would give a totality which would have no affinity, no attraction, for the male element. In fact, the zoösperms do not advance toward the interior of the vesicle so long as the latter remains intact. The eliminated substances should, by this hypothesis, have a polarity of the same name as that of the zoösperm, or the same chemical affinities. One could then understand how it is that the presence of a zoösperm in the vitellus hastens the elimination of the polar globules. On the other hand, the penetration of a zoösperm into a polar globule — a fact which has been once or twice observed — would remain inexplicable. The cause of the obstacle which it seems to offer to fecundation would be seen in its size and inactivity. The expelled portion would be the more passive, the female pronucleus the more active principle.

Even if in fecundation it is evident that a zoösperm exercises an influence upon the vitellus from which it is still separated by a relatively large space, the mechanism of that action is not clear. The author sees only three hypotheses which can accord with the facts. The zoösperm may be separated only in appearance; there may be a continuity of sarcodic substance as soon as the action is exerted. But as he has found no filament of sarcode extending from the zoösperm toward the vitellus, and no change in the form of the body of

the former, such as must result if such a filament exists, it only remains to assume pre-existing filaments which arise from the surface of the vitellus. These might, *a priori*, be represented as extremely delicate filaments traversing the oölema in radial lines; but as they have never been discovered in the hardened or living egg, it is necessary to deny their existence. The second hypothesis is that the action of the vitellus is in response to a pressure exerted by the zoösperm upon the intervening portion of the striate envelope through which it endeavors to advance. As the vitellus does not react upon the pressure of all kinds of bodies, it is necessary to admit that there is some peculiarity, — some special rhythm arising from the undulations of its cue. But it is difficult to understand how this pressure could be appreciated through half the thickness of the oölema, or communicated to a definite extent of the vitelline surface, or why the cone of attraction should arise exactly opposite the most advanced zoösperm. The last supposition consists in admitting an attraction, the nature of which is unknown, which exercises an influence not only by immediate contact, but also at a slight distance; although this hypothesis itself needs to be explained. The composition of the cone of attraction is not better understood. Is it a substance secreted by the vitellus, or a prolongation of the vitelline sarcode, and, in the latter case, is it an accumulation of the superficial limiting layer, or of the deeper layer? The first hypothesis is excluded, in the author's opinion, by the case where the protuberance is of considerable volume, and the continuity of its substance with the vitelline sarcode is evident, but between the other two hypotheses he remains undecided. The cone of *exudation*, on the other hand, is only a liquid, slightly refringent substance, without cohesion, which is ejected or excreted by the surface of the yolk.

Fol distinguishes three kinds of centres of attraction, — the male, the female, and those which preside at segmentation. The male centre takes its origin in a spermatozoön,* whose "*body*" *becomes the centre of an aster* and the point of departure in the formation of the male pronucleus. I am not certain what he means by that part of the statement which I have italicized, since he has shown more clearly than any one else that the male aster does not always *centre* at the middle of the male pronucleus. I can reconcile this statement with the fact mentioned only by assuming, either that he thinks the male pronucleus is formed eccentrically to the head of the spermatozoön, or that this statement is only intended to be an *approximate* expression of facts. The former assumption is the less probable, because he adds: "It is important not to forget that the body of the spermatozoön is no longer intact at the moment when these phenomena (astral) appear; it has changed form and has increased in size by the absorption of vitelline sarcode." I therefore think the

* The author thinks recent observations tend to show that the spermatozoön is formed of cellular protoplasm, *to the exclusion of the substance of the nucleus.* Consequently the male pronucleus is formed by the union of two protoplasms, which have not suffered a single admixture of the substance of preformed nuclei. "Le pronucléus mâle ne descend à acun titre, pas même en partie, d'un noyau plus ancien; il est de formation nouvelle."

author has attached far too little importance to his observations on Sagitta (Pl. X. Fig. 6). He remarks that the attraction is therefore exercised not so much by a simple spermatozoön as by a fusion of this with the sarcode, and that it is this union which gives rise to the male pronucleus. This statement approaches so closely the view I have already advocated, that I should be inclined to think our ideas on this point identical, were it not that he subsequently explains his position in a manner which shows clearly that in his opinion it is the *substance of the male pronucleus* which exercises the attractive influence rather than a force liberated in the *act of the union.* He says, substantially: "The male center is surrounded soon after its formation by a star of unipolar rays. Shortly the centre, represented by the body of the more or less modified spermatozoön, is surrounded with clear protoplasm. This mass continues to increase, — a fact which seems to indicate that the sarcodic rays are the expression of centripetal currents of protoplasm coming from the vitellus. However that may be, it is certain that the aster is formed around a modified spermatozoön which is found at its centre; that it is a result of *the action exercised by this corpuscle upon the surrounding vitellus.* If this is so, it should be explained why the evidence of this attraction ceases when the nucleus has attained its maximum size.

The phenomena of attraction, Fol continues, are perhaps less striking for the female than for the male pronucleus, but they exist none the less. They are, — (1.) radial lines, which continue to augment in proportion as the pronucleus absorbs vitelline sarcode, and are only effaced at the moment when it has come to rest; (2.) the centripetal advance of sarcodic currents, of which the radial striæ are the visible expression, and the direction of which is indicated by the growth of the nucleus; (3.) the displacement of the pronucleus itself from the periphery toward the centre of the yolk.

The centres of attraction which appear at the poles of the amphiaster of segmentation are due to a fusion (rencontre et alliage) of nuclear substance with vitelline sarcode at the circumscribed points (poles) where the contour of the elongated nucleus becomes lost. But this is not the first process preliminary to the formation of the amphiaster. On the contrary, in the case of the sea-urchin the appearance of a mass of sarcode around the nucleus, as well as the "pinnate figure," precedes. The latter appears to be the expression of *centrifugal* rather than centripetal currents; the formation of typical asters, on the other hand, only dates from the moment when the nuclear and vitelline substances enter into communication at the poles of the nucleus. The three cases have in common this point: that the phenomena of attraction (and of repulsion) may precede the mingling of two diverse substances, but that they attain their full development, and interpret themselves by the formation of a veritable aster, only when there has been a fusion of the two substances; the point of fusion is then always the centre of the system of rays. It seems to me this last statement is more in harmony with the view I have maintained than it is with the ideas which Fol has himself previously advocated. In my opinion it is the *point* of fusion, and not necessarily the *product* of the fusion,

which marks the centre of attraction ; it is the force set free in the *act* of fusion, not the affinity of the already formed nucleus for other matter, which induces the radiate appearance. If this view is justified, it seems to me that it would not be necessary to make a special case of Sagitta, and to hesitate, as Fol does (p. 259), in comparing the structure which he there calls by the non-committal name "la vacuole" with the male pronucleus of other animals.* I have less hesitation in pronouncing this "vacuole" in every essential the equivalent of the male pronucleus of other animals, as I have seen something so nearly identical in Limax (see Fig. 68), where the staining of the structure in question banishes all doubt as to its nuclear character. I must confess, moreover, that I am considerably puzzled to know what Fol means by this hesitancy, as I see no other possible explanation in view of the ultimate fate of this vacuole. Instead of presenting any difference of primary, or even secondary importance, it seems to me a very welcome confirmation of the substantial identity of the astral phenomena which accompany the male pronucleus and those which preside at the subsequent segmentations.†

In regard to the nature of the rays, the author says, the hypothesis of a simple polar attraction which arranges the vitelline granulations in a certain order without displacing them is not defensible ; for these bands are in all cases broader than the mean distance of the lecithic granules. These filaments, so distinct in acetic acid, do not admit the belief in a simple polarization of molecules. The accumulation of clear protoplasm around the nucleus and its poles could not take place without currents of this viscid substance. But if there are currents, in what direction are they produced? In the sea-urchin, before the formation of the amphiaster, the perinuclear mass moves toward the equator, and becomes a disk at the moment when the pinnate arrangement of the clear streaks becomes visible. Here it may well be that the currents arising in the equatorial region proceed beyond the poles to spread their substance in the vitellus. During the division of the amphiaster the facts appear to favor the supposition of centripetal currents.. The centripetal movement of the thickenings [in the unipolar rays] which are rendered visible by acetic acid, and the continuous growth of the central masses, appear to indicate a slow advance of the sarcode toward the centre of the aster.

Nevertheless, observations on other objects must, he says, be taken into the account. Auerbach's theory of a dispersion of nuclear fluid is untenable, since the asters are formed before the volume of the nucleus is diminished. On the other hand, Flemming has seen pseudopodia at the surface of the polar globule in Anodonta, of which the following explanation is offered. From the figures

* "Chez Sagitta, par exemple, nous avons vu que l'aster traîne à sa suite une sorte de vacuole toujours croissante ; le centre de l'aster se trouve au bord allongé de cette vacuole, toujours du côté vers lequel elle se dirige. C'est donc dans l'aster et dans son centre que réside la force motrice, tandis que la vacuole, que j'hésite du reste à comparer au pronucléus des autres animaux, est traînée à sa suite."

† P. S. — May it not be that this is a typographical error, and that the *negative* has been inadvertently omitted?

(Flemming's) it is to be seen that the polar globule is half formed at this time, and consequently "Bütschli's corpuscles" are already divided. As is known, the external aster is incomplete, its centre having arrived at the surface and being only partly surrounded by unipolar filaments. It is therefore natural to presume, he says, that the pseudopods correspond to unipolar filaments, which are wanting in consequence of the superficial position of the aster. If this reasoning is correct, he adds, this is a case where the filaments are elongated in a centrifugal direction during a part of the period of division, to be subsequently retracted. If the pseudopodia belonged to a somewhat earlier phase, I should think there would be greater justice in this conclusion. The position of the rays after the division of the equatorial zone, as shown in Limax, Fig. 50, does not seem easily reconcilable with Fol's interpretation.

But the observations of Strasburger on Spyrogyra also tend, continues the author, to establish the existence of centrifugal currents during the division of the amphiaster. If, now, we compare the unipolar filaments with these pseudopodal filaments (Spirogyra), the former should be considered as streaks of sarcode stretching out toward the periphery, but having the opposite course after the division of the intranuclear enlargements.

The changes within the nucleus are also instructive. The formation of the spindle from the intranuclear network and the division and migration of the fibre thickenings appear to result from an action exercised upon the interior of the nucleus by two centres placed at its poles. It is not possible to say what the nature of this action is. An internal repulsion would not explain the eccentric position of the "amphiaster de rebut." A simple attraction would not explain the formation of Bütschli's corpuscles. On the other hand, the last part of the act of division, the formation of new nuclei, appears explainable upon the hypothesis of an attraction exerted by the centres upon their vicinage, and of the mutual repulsion of the asters. A central attractive influence on the part of the new nuclei would explain the process of segmentation, except in the case of the polar globules.

The evidences of the existence of repulsions are the progressive separation of the poles of the amphiaster and the mutual repulsion of the male asters. After the female pronucleus has been superfecundated the conjugated nucleus and the independent male asters are all situated at the external third of vitelline rays. This regularity of situation indicates that they occupy a position of equilibrium between opposing forces, and these forces can only be an attraction toward the centre of the yolk on the one hand, and a mutual repulsion on the other. The attraction between the sexual nuclei is a special case in which this force is very evident. The motion of the male aster is correlated with the position of the female pronucleus; it has not a constant relation with the vitellus.

In the further development, the male elements exercise a preponderating influence, as the existence of the tetraster after fusion with more than one zoösperm shows; the isolated male asters pass through the stages of amphiastral division, but the isolated (unfecundated) female pronucleus decomposes without exhibiting any of these changes.

The male pronucleus arises when a living zoösperm penetrates into a mature and living vitellus. In certain cases (sea-urchin) it is not much larger than the "body" of a zoösperm, and one might then entertain the opinion that it is only such a "body" swollen. It then forms the centre of an aster. In other cases (Heteropoda) it becomes as large as the female pronucleus, and is not surrounded by a radial figure.* This growth is not a simple inflation of the body of a zoösperm by a liquid, because when fully developed it contains many times the original quantity of protoplasmic substance ; it is not a process of nutrition, a digestion of vitelline substance, since that physiological process is complicated and requires a considerable time for its accomplishment, while the absorption of vitelline substance is direct and prompt. The male pronucleus is therefore a product of the union of spermatic protoplasm with vitelline protoplasm, and from this fusion results a nuclear body possessing a multitude of properties which are wanting to the isolated zoösperm.

Likewise the female pronucleus, which has its first origin in the "corpuscules de Bütschli" belonging to the internal half of the second "amphiaster de rebut," is derived for the greater part from vitelline sarcode. The disproportion between nuclear and vitelline substances in this case is fully as great as between the body of the zoösperm and the completed male pronucleus in the Heteropoda.

Finally, the cleavage nuclei are formed at the expense of the "central masses" and of the intranuclear varicosities of the old nucleus. These "masses" may also descend, in part at least, from the old nucleus. But even here the substance derived from that source is only a fraction of the whole mass of the new nuclei. Besides, a part of the substance of the old nucleus remains *en route* under the form of the "trainée internucléaire," and does not enter into the composition of the new cytoblasts.

A consideration of the origin of the nucleus in these three cases leads to the same conclusion, that its substance comes in part from a pre-existing nucleus or a foreign element, and in part from the protoplasm of the cell, — the latter by way of fusion, not of nutrition. The young nucleus while still quite small exercises a strong influence on the surrounding vitellus. In proportion as it increases in size, this influence diminishes, and once completed, it ceases to exist. It therefore seems permitted to conclude that the attraction or influence exercised by the young nucleus increases in proportion as it is fused with cell protoplasm, and subsequently diminishes when the proportion of the latter element is too great. There would be a period of activity followed by a period of saturation ; the latter would supervene as soon as the nucleus attained the limit of its growth.

I can omit a presentation of the author's "Théorie électrolytique des Mouvements protoplasmiques," with which he terminates his paper, since it is not directly applied to an elucidation of the phenomena under consideration.

* The author has previously stated, I believe, that in the Heteropoda the male pronucleus is surrounded by rays in the living egg. See p. 577.

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EXPLANATION OF FIGURES.

LETTERS.

The following letters are used throughout to designate respectively:—

A. *A* = Amphiaster.
A[1] = First archiamphiaster.
A[2] = Second archiamphiaster.
A[3] = Amphiaster of first segmentation sphere.
a = Aster.
aa = Central area of aster.
aa′ = Structures at the centre of *aa*.
ae = External aster.
ai = Internal aster.
ar = Rays of aster.
ar′ = Thickenings in *ar*.
ars = Spiral aster-rays.

F. *fpn* = Female pronucleus.
fpnl = Female pronucleolus.

M. *ma* = Male aster.
mpn = Male pronucleus.
mpnl = Male pronucleolus.

N. *n* = Nucleus.

P. *p* = Pedicel, or neck of polar globule.
pn, pn′ = Pronuclei.
pnl = Pronucleoli.
pp = Pedicel-plate (Zellplatte?).
Pv = Purkinjean vesicle.
pz = Clear zone in the yolk.

R. *r* = Polar globule (Richtungsbläschen).
r′ = First polar globule.
r″ = Second polar globule.
rn = Nuclear structure of *r*.
rnl = Nucleolar structure of *r*.

S. *sp* = Nuclear spindle.
sp[1] = First maturation spindle.
sp[2] = Second maturation spindle.
sp[3] = Spindle of first segmentation sphere.
spf = Spindle fibres.
spf′ = Interzonal filaments.
spf″ = Thickenings in *spf′* (Zellplatte?).
spl = Lateral zones of spindle thickenings.
spm = Median (equatorial) zone of spindle thickenings.
spz = Spermatozoa.

V. *V* = Vitellus.
ve = Vitelline envelope.
vm = Vitelline membrane.

TREATMENT, ETC.

All Figures, except 62ª, 94, and 95, were drawn with the aid of the Chevalier-Oberhäuser camera; and all, except 80ᵇ, 80ᶜ, and 95, relate to *Limax campestris.*

Figures 1–21, 27, 49, 51, 62ª, 65, 70ª, are *magnified* **140** diameters; Figs. 30–38, 76, about **200** diameters; Fig. 95, **300** diameters; all other Figures **750** diameters.

The following Figures were made from *living* eggs: 1–21, 27, 30–38, 49, 51, 62ª, 65, 70ª, and 95.

Figures 28, 29, 44, 52, and 52ª, are from *sections* of eggs hardened in *chromic* acid; all others, except Figs. 26 and 76, from the egg entire.

Osmic acid (1%), followed by carmine, was employed to harden and stain those from which Figs. 63, 64, 68–70, 71, 72, 75, and 77, were drawn. Those of Figs. 69 and 75 remained unstained by Beale's carmine.

All eggs not otherwise specified were treated with *acetic* acid (1%–2%) for three hours or more, and were subsequently stained in Beale's carmine.

PLATE I.

Figs. 1–20. Formation of polar globules.

Figs. 1–9. Successive views of the same egg at 6:00, 6:02, 6:04, 6:06, 6:30, 7:40, 9:30, 10:45, and 11 o'clock.

Figs. 10–14. Formation of second polar globule. Another egg seen at 11:08, 11:09, 11:10, 11:12, and 11:15 o'clock.

Figs. 15–20. Formation of second polar globule as seen in another egg, at 6:08, 6:33, 7:04, 7:05, 7:06, and 7:15. First segmentation of this egg nearly completed at 10:00 o'clock.

Fig. 21. Egg showing an irregular zone of clear protoplasm and two pronuclei.

Fig. 22. The deeper of the lateral zones of fibre thickenings has reached the border of the well-defined central area of the internal aster. Optical meridional section.

Fig. 23. Optical section in the plane of the polar globule, showing the second archiamphiaster; the peripheral aster more sharply outlined than the deeper one.

Fig. 24. Same seen along the axis of the spindle. Focused a little above the centre of the superficial aster; the polar globule "projected." (Its outline has been made too irregular and ragged in lithographing.)

Fig. 25. Another egg of about the same stage, and seen in the same position as Fig. 22.

Fig. 26. Yolk elements from the vitellus of a crushed egg.

Fig. 27. Peculiar appearance, as of decussating fibres, seen at the animal pole after the formation of the first polar globule.

Figs. 28, 29. The fourth and third respectively of five successive sections of the egg, Fig. 21, put in acid during the first segmentation. Slightly distorted by the traction of the knife in cutting. The plane of section is not quite parallel with the plane determined by the polar axis and the line joining the centres of the two asters, but cuts *both* these lines. From its obliquity to the polar axis, it results that the polar globules, and the curved remnant of the spindle (interzonal filaments), which both lie in this axis, are found not in the same but in successive sections. From its obliquity to the line joining the astral centres, it results that the nucleus of one of the two segmentation spheres is cut, as shown in Fig. 29, while the other remains untouched, and also that the interzonal filaments (*spf''*) are cut across in the sphere marked "*y*" (Fig. 29). Chromic acid preparation.

Figs. 30–32. Three successive views of an egg, at 8, 8:54, and 9 o'clock.

Fig. 30. Polar globules already formed. The two pronuclei with very clear circular outlines of nearly equal size. No change from the spherical form observed.

Fig. 31. The egg has changed form slightly, and two oval, ill-defined spots are visible, at some distance apart, the pronuclei having disappeared. The yolk shows a faintly expressed radial arrangement of granules about these two spots.

Fig. 32. The spots are farther apart; the radiate arrangement more distinct; the cleavage furrow at the animal pole of the yolk is quite pronounced.

Figs. 33–35. Three views of an egg, at 7:54, 8:35, and 9:12 o'clock.

Fig. 33. A clear spot at the animal pole, and deeply penetrating narrow zone of clear protoplasm (*pz*) near the equator.

Fig. 34. The clear spot has moved to near the centre of the egg; the equatorial zone is less distinct.

Fig. 35. The first segmentation furrow has already extended to the vegetative pole.

Figs. 36–38. Three views of the same egg, at 8:10, 9:04, and 9:15 o'clock.

Fig. 36. Male and female pronuclei, and equatorial clear zone (*pz*) visible, the latter intermediate in prominence between that of Figs. 33 and 34.

Fig. 37. Beginning of the first segmentation of the yolk. The pronuclear structures have disappeared, and the oval spots, from the position of the egg, partly cover each other.

Fig. 38. Near the close of the first segmentation.

PLATE II.

Fig. 39. Optical section of an egg, showing the first archiamphiaster, and peripheral clear areas in the yolk.

Fig. 40. Optical section of an egg and first polar globule, with lateral zones of thickenings, prominent interzonal filaments, and possible indications of an amœboid character of the yolk at the animal pole.

Fig. 41. Surface view of the polar globule of the same.

Fig. 42. View of same globule, the optical axis coinciding with the primary (animal) radius of the egg. It shows the annular arrangement of the spindle thickenings, and the outline of the pedicel.

Fig. 43. First archiamphiaster. The external aster causes a protuberance at the surface of the yolk, and exhibits a highly refractive body at the centre of radiation. Nuclear spindle inconspicuous; equatorial thickenings not prominent.

Fig. 44. Section of an egg just before the first segmentation. The plane of section is parallel with the axis of the spindle. About one third of the spindle was cut away by the section preceding the one here represented. A highly refractive spherical body occupies the centre of each of the astral areas, and the thickenings of the spindle fibres are arranged in two closely approximated parallel zones.

Fig. 45. First archiamphiaster at the time the rays of the external aster attain the surface of the yolk. The external aster is more sharply outlined than the internal. The first maturation spindle presents only a single (equatorial) zone of fibre thickenings. The flattened appearance of the animal pole is probably due to the resting of the yolk on that pole during its preparation, and while still incompletely hardened.

Fig. 46. Equatorial optical section of the same; the spindle thickenings are projected, and exhibit the usual annular arrangement.

Fig. 47. External aster of same; the optical axis coinciding with the axis of the spindle. The rays have a spiral course.

Fig. 48. The first archiamphiaster has approached the animal pole still more closely than in Fig. 43. The external aster has thereby become more conspicuously unsymmetrical. The spindle is more distinctly marked, and shows the equatorial zone just dividing into its lateral halves. Both asters exhibit large areal corpuscles. The "halos" around each should be narrower; more as in Fig. 43.

Fig. 49. Living egg at the close of the formation of the first polar globule. Radi-

ate markings at the clear animal pole of the yolk. Numerous highly refractive spermatozoa in the vicinity of the vitellus.

Fig. 50. First archiamphiaster has migrated still farther than in Fig. 48 toward the animal pole, the centre of the external aster having nearly attained the surface of the yolk. The conical protuberance caused by *ae* is covered by a cap of finely granular substance (compare text, p. 198). Lateral zones of spindle thickenings half-way between the equator and the poles of the spindle.

Fig. 51. A single ill-defined clear spot seen in the living egg, where the pronuclei are found in the same egg hardened and cut (Figs. 52, 52^a). Equatorial zone of clear protoplasm, *pz*.

Figs. 52, 52^a. The third and fourth of five sections through the egg, shown in Fig. 51, the egg having been put in chromic acid immediately after the outlines (Fig. 51) were made. Both pronuclei contain numerous nucleolar bodies joined by irregular fibres, which thus produce an indistinct reticulum.

In Fig. 52 are to be seen the two polar globules, nearly the whole of the female pronucleus, a portion of the male pronucleus, and, near the border of the latter, an incipient aster of A^3, with a conspicuous highly refractive structure (*aa′*) occupying the centre of the astral area.

In Fig. 52^a is seen the remainder of the male pronucleus. No other aster had as yet made its appearance in the yolk.

PLATE III.

Fig. 53. Archiamphiaster, whether the first or the second is uncertain. Consult text at pp. 189, 206.

Fig. 54. Another view of the same. The optical axis coincides with the axis of the incipient spindle. The nuclear substance, largely accumulated on one side of the spindle, is seen as though "projected" on the equatorial plane passing through the centre of the internal (deeper) aster.

Fig. 55. Second archiamphiaster. The spindle exceptionally slender. The yolk about the animal pole is constricted by two or three rings, which give it a wavy outline when seen in optical section. Compare with the description of the formation of polar globules in Clepsine, as given by Whitman (**'78**^{a}, pp. 232, 233, and *separate*, pp. 18, 19, Pl. XII. Figs. 2–6).

Fig. 56. Equatorial optical section of same. The external aster with spiral rays projected; optical axis slightly inclined from the spindle axis.

Fig. 57. Internal aster of the second archiamphiaster, with compound curvature of rays. Male and female pronuclei; the centre of the aster nearer the latter. "Interzonal filaments," exceptionally prominent, unite the second polar globule to the vitellus. A thin pellicle (vitelline membrane?) stretches over the second polar globule, the first having become detached. Granulations of the yolk omitted.

Fig. 58. Male and female pronuclei, the latter near, but not coinciding with, the centre of the inner aster of the second archiamphiaster. In this figure only that portion of the inner aster is shown which lies very near the surface of the yolk, the rays of which are stout and nearly straight. The centre of the astral area is occupied by a few granules not quite so conspicuous as the pronucleoli, and certainly not embraced within the outline of the female pronucleus, which is well marked. Compare Fig. 78 or deeper portions of this aster.

Fig. 59. Yolk of exceptional form. The primitive axis lies in the plane of the optical section. The pronuclei, male and female, have attained considerable size, the latter still united to the polar globule by interzonal filaments. About midway between the pronuclei a dense, but less granular area, around which the yolk granules show a radial arrangement, — the senescent internal aster of the second archiamphiaster. The region of this aster is more deeply stained than the surrounding yolk. Another region of crescentic form appears in the vegetative half of the yolk beyond the male pronucleus, and is likewise deeply stained. It is represented in the figure by deeper shading.

Fig. 60. Optical section oblique to the primitive axis. Polar globule "projected." Male and female pronuclei, the latter distinct from the central area of the internal half of the second archiamphiaster.

Fig. 61. Equatorial optical section. Polar globules, pedicel, lateral zone of thickenings, granules of astral area, and short stout rays of aster, projected. Granulation of the yolk omitted. A sufficient difference in the prominence of the long and the short rays has not been observed in lithographing.

Fig. 62. Meridional optical section of same, the first polar globule being omitted. Lateral zone and areal corpuscles more nearly approximated than usual.

Fig. 62[a]. Living egg near the close of the first segmentation. Consult the text at p. 223.

Fig. 63. Female pronucleus small, homogeneous, lying at the border of the central area of the internal aster. The "interzonal filaments" exhibit a plate, *pp* (the Zellplatte ?), near the point of their deepest constriction. The areal corpuscle of the external aster fused with the envelope of the polar globule at its distal pole. Osmic acid preparation.

Fig. 64. The second polar globule of the same egg seen from the animal pole. Osmic acid preparation.

Fig. 65. The male and female pronuclei in the living egg.

Fig. 66. Formation of the second polar globule; "interzonal filaments" bent nearly at right angles (compare Fig. 19); the spiral rays of the internal aster radiate from a spiral line, $\alpha\beta$; the areal corpuscles and the thickenings of the internal zone not distinguishable from each other; two vesicular structures in the vegetative hemisphere — incipient male pronuclei (?) — do not contain nucleolar corpuscles.

Fig. 67. Portion of the same egg seen after rotating the yolk 90° about the primitive diameter as an axis. The internal end of the spindle is deeply stained, but not sharply defined.

PLATE IV.

Fig. 68. Second polar globule with nucleus, and longitudinal folds in the envelope of its pedicel. The position of the vitelline half of the "interzonal filaments" is indicated by a streak of non-granular protoplasm extending to the female pronucleus, in which, however, filaments are not traceable. Both pronuclei pear-shaped, with the sharper ends (not outlined with sufficient distinctness) directed toward the centre of a clear spot which is surrounded by numerous faint rays, — the senescent internal aster of the second archiamphiaster. The pronucleoli more numerous in the female (25) than in the male (20) pronucleus. Osmic acid preparation.

Fig. 69. Oblique view of the formative pole of the yolk (the two polar globules

omitted), showing male and female pronuclei, in which no nucleolar structures are discernible. An invagination of the yolk into one side of the female pronucleus is compensated by an evagination of the wall of the latter into the substance of the male pronucleus. Compare Fig. 75. Osmic acid preparation.

Fig. 70. Pronuclei. The position of the internal aster of A^2 is indicated by the irregular non-granular area near the female pronucleus, but no radial differentiation can be distinguished. The pronucleoli of about the same number (15 and 16) in each of the pronuclei. Osmic acid preparation. Compare Fig. 72.

Fig. 70ª. Living egg. Recession of the granular yolk from the surface, especially at the primary pole, which lies a little to the left of the polar globules.

Fig. 70ᵇ. Early stages in the formation of the pronuclei: α, the female pronucleus; β, the male.

Fig. 71. Second polar globule of the egg shown in Fig. 72 with two nuclear structures, as seen after rotation about the primitive diameter as an axis. Osmic acid preparation.

Fig. 72. Meridional optical section. The pronuclei large, considering the distance between them. The centre of the senescent internal aster of A^2 nearly coincident with the centre of the female pronucleus, as shown by the course of the faint rays still traceable. Osmic acid preparation. See also Fig. 70.

Fig. 73. Pronuclei still unconsolidated after the appearance of *one* of the asters of the amphiaster of the first segmentation sphere. Nucleoli numerous. No trace of the complementary half of this amphiaster discoverable. Compare Fig. 80.

Fig. 74. Both asters of A^3 extensively developed; one distant from the female pronucleus, which still remains unfused with the male pronucleus, although in contact with it.

Fig. 75. A deep cup-shaped invagination of the yolk has forced inward one side of the female pronucleus, and a slight projection from the opposite side of the latter is plunged into the male pronucleus. Both present a wrinkled appearance, but no trace of nucleolar structures. Osmic acid preparation. Compare Fig. 69.

Fig. 76. Yolk crushed after the formation of the amphiaster of the first segmentation sphere. The spindle is proportionately somewhat shorter than before the yolk was crushed. Slender strings of protoplasm stretch from the spindle to one of the fragments of an aster.

Fig. 77. The two pronuclei near the primary pole, each containing about a dozen nucleoli. Those of pn' are shaded to distinguish them from those of pn. No trace of either aster. Osmic acid preparation.

Fig. 78. Equatorial optical section of the egg shown in Fig. 58. The outlines of the polar globule and of the two pronuclei "projected." The superficial rays and the granulations of the yolk are omitted, so as to show better the spiral course of the numerous deep rays. Seen from the primary (animal) pole. Consult text, p. 209.

Fig. 79. Pronuclei seen from the primary pole, each containing about thirty pronucleoli. Only one aster of A^3 discernible. Pronuclei not confluent.

Fig. 80. The egg which is exhibited in Fig. 73, so rotated that the face of the pronucleus nearest the aster appears in profile. The centre of the aster lies at some distance from the sharp outline of the pronucleus.

Fig. 80ª. Egg near the close of the second segmentation. The outlines of two of the blastomeres, and partial outlines of the other pair, as seen from the secondary

pole. Compare the shape of the nuclei, and the relation to their respective asters, with that of the pronuclei in Fig. 68. The more pointed ends of the nuclei are directed obliquely away from the observer, and the interzonal filaments, which are much thicker in the middle than toward the ends, are so bent as to present to the observer their convexities.

Fig. 80^b. Nearly meridional view of the "primary" half of an egg from an undetermined species of Limax. Each pronucleus contains a single nucleolar structure which greatly exceeds any of the others in size; it is indicated by its shading, the remaining nucleolar bodies being only outlined.

Fig. 80^c. The second polar globule of the same egg as that last figured, seen in profile, to show the relation of the vitelline membrane, detached by the hardening reagent, and the interzonal filaments to both polar globule and yolk.

Fig. 81. Vitellus showing one extensive aster with a homogeneous centre (female pronucleus?), the rays of which are numerous and slender, and several other less extensive asters with few stout rays. The latter are probably induced by the penetration of a corresponding number of spermatozoa into the yolk. Abnormal condition.

PLATE V.

Fig. 82. Third amphiaster (A^3) after the almost complete disappearance of the pronuclei. A few exceedingly faint outlines (*pnl*?) may possibly be traces of pronucleoli. An irregular plane of prominent granules (incipient nuclear plate?) separates the halves of the amphiaster. Asters flattened in the direction of the axis of the still incomplete spindle. Thickenings (ar') occur near the central ends of many of the rays. The central areas contain numerous prominent granules (aa'). Compare with Fig. 85.

Fig. 83. Amphiaster of the first segmentation sphere, with very prominent spindle and equatorial zone of fibre thickenings (*spm*), the latter shown in

Fig. 84, as they appear when the optical axis corresponds with the axis of the spindle.

Fig. 85. View from the *secondary* (vegetative) pole. The asters of A^3 well developed before the complete union of the pronuclei. A few highly refractive granules near the axis of the future spindle (too prominent in the engraving). One of the astral areas is homogeneous, the other contains granules. The rays of the asters present thickenings forming a zone concentric with the area. The external limits of the thickenings are not sufficiently defined. The zones, interrupted two or three times by the absence of thickenings from several neighboring rays, are not quite accurately reproduced.

Fig. 86. A nearly face view of the amphiaster of the first segmentation sphere. A remnant of the substance of the pronuclei is still visible between the spindle and the animal pole.

Fig. 87. The egg shown in Fig. 86, seen lengthwise of the spindle. The remains of the nucleus are more distinct than in the preceding view. No fibre thickenings (nuclear plate) observed. Compare also Figs. 88 and 89.

Fig. 88. A slightly more advanced stage than is shown in Figs. 86, 87. The asters nearly cover each other, the line of vision being almost parallel with the spindle axis. The remnant of the nucleus, still sharply outlined, lies near the animal pole, and is surrounded with a narrow zone of non-granular protoplasm.

Fig. 89. The same egg as the last, rotated nearly 90° about its primitive axis. The remnant of the nucleus appears less sharply outlined. The elements of the equatorial nuclear plate are very evenly arranged, and conspicuous.

Fig. 90. A slight furrow introducing the first segmentation of the yolk has made its appearance at the animal pole. A vitelline membrane is detached from the yolk over a space corresponding to this furrow. The nuclear spindle is viewed somewhat obliquely, so that the lateral disks of fibre thickenings are not seen exactly edgewise, and therefore appear oval. The left-hand edge is represented in the lithograph as farthest from the observer; the *right-hand* edge of the oval should have been made the fainter, as it is really the more remote.

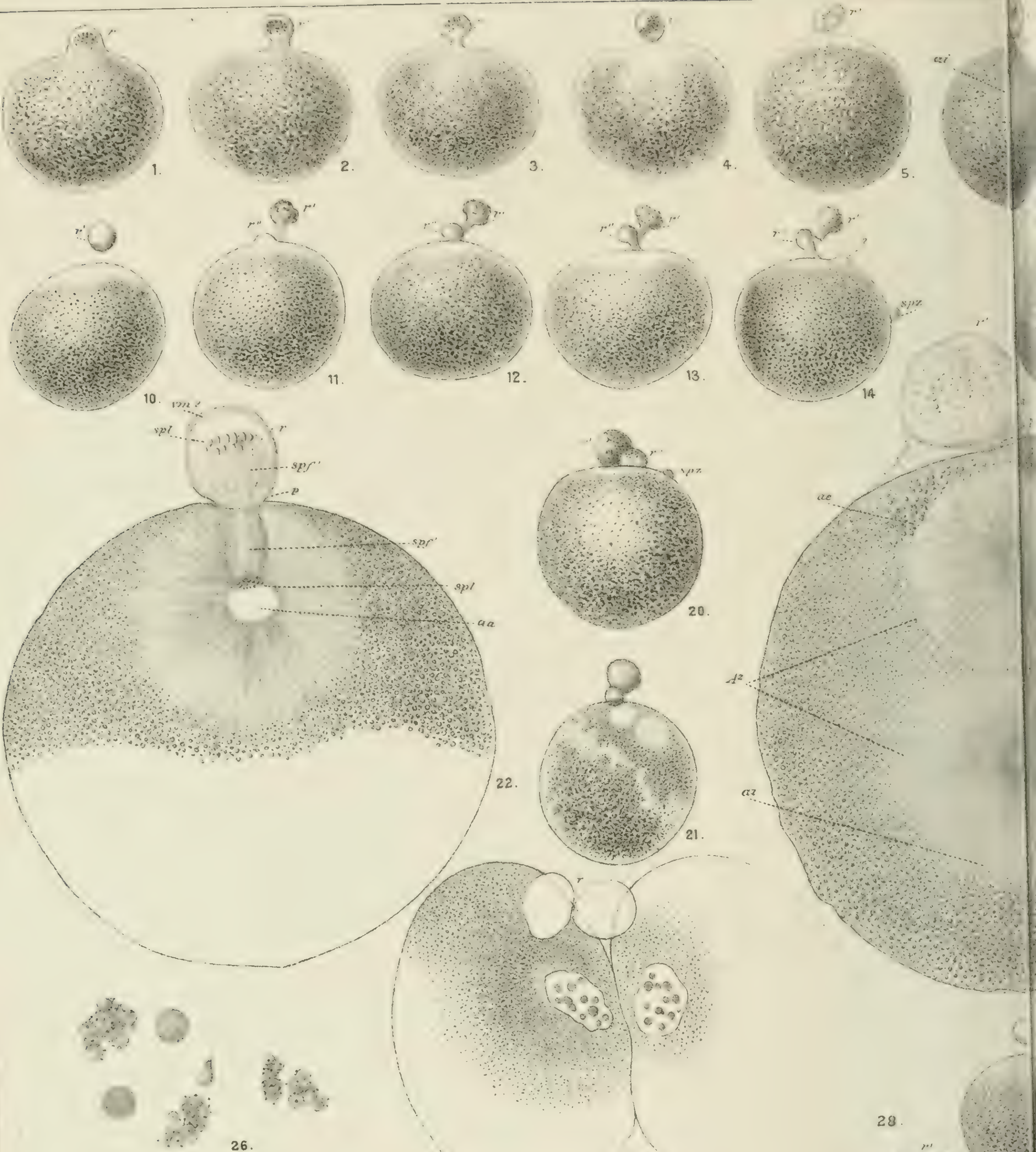
Fig. 91. Constriction further advanced than in Fig. 93; nuclei much larger; interzonal filaments distinguishable only near the plane of division between the two secondary cells. Asters becoming less distinct.

Fig. 92. Equatorial thickenings in the spindle of the first segmentation sphere, as seen when the spindle is viewed lengthwise.

Fig. 93. Formation of the nuclei of the first pair of blastomeres. Interzonal filaments sharply bent and slightly thickened. Compare Fig. 29.

Fig. 94. Spermatozoön with vibratile (?) membrane. The tail end should have been made thinner. The free edge of the membrane indicated by the sinuous line.

Fig. 95. View of a portion of a living egg of Limax sp.? toward the end of the formation of the second polar globule, to show the existence of pseudopodia.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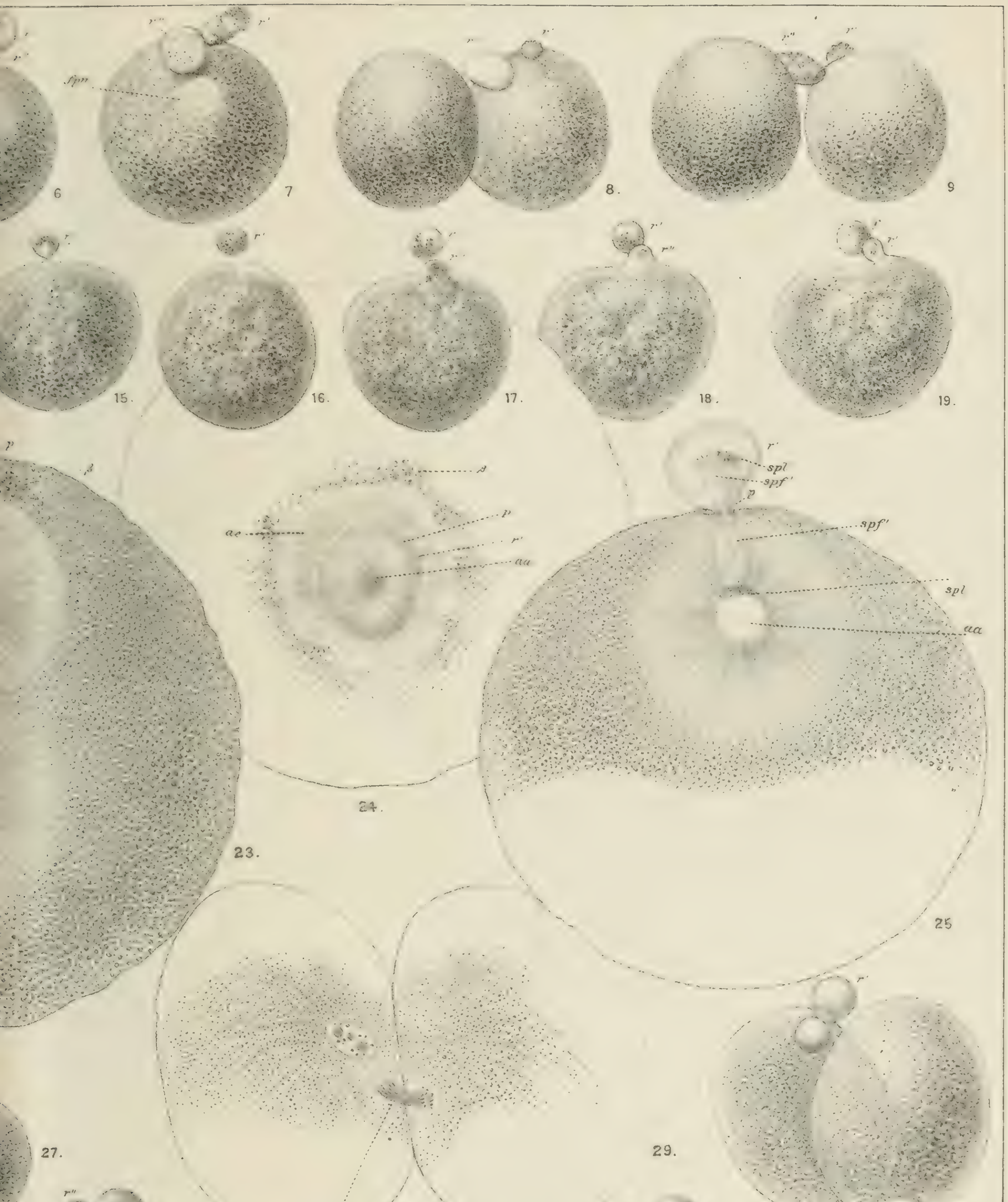

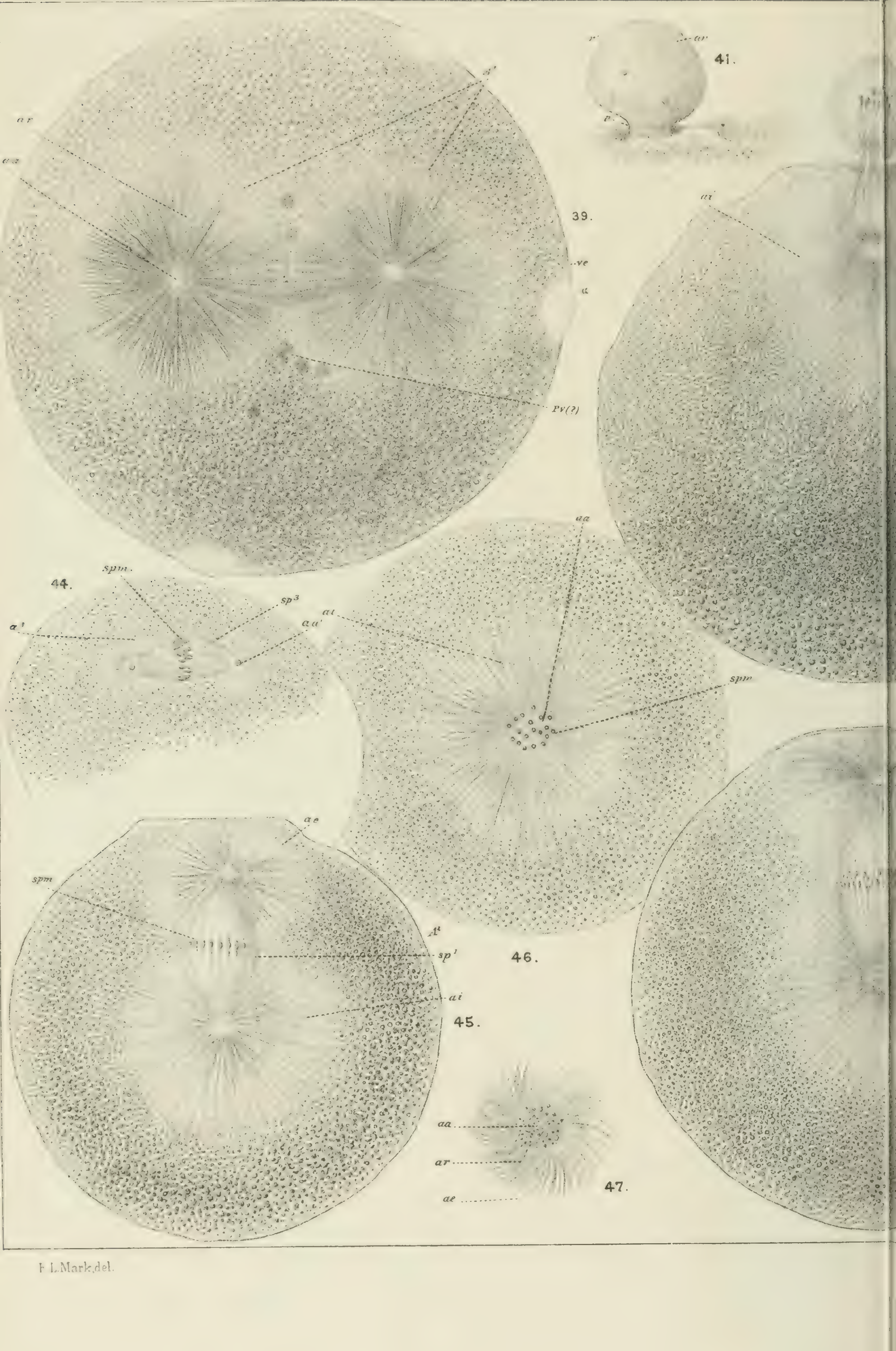

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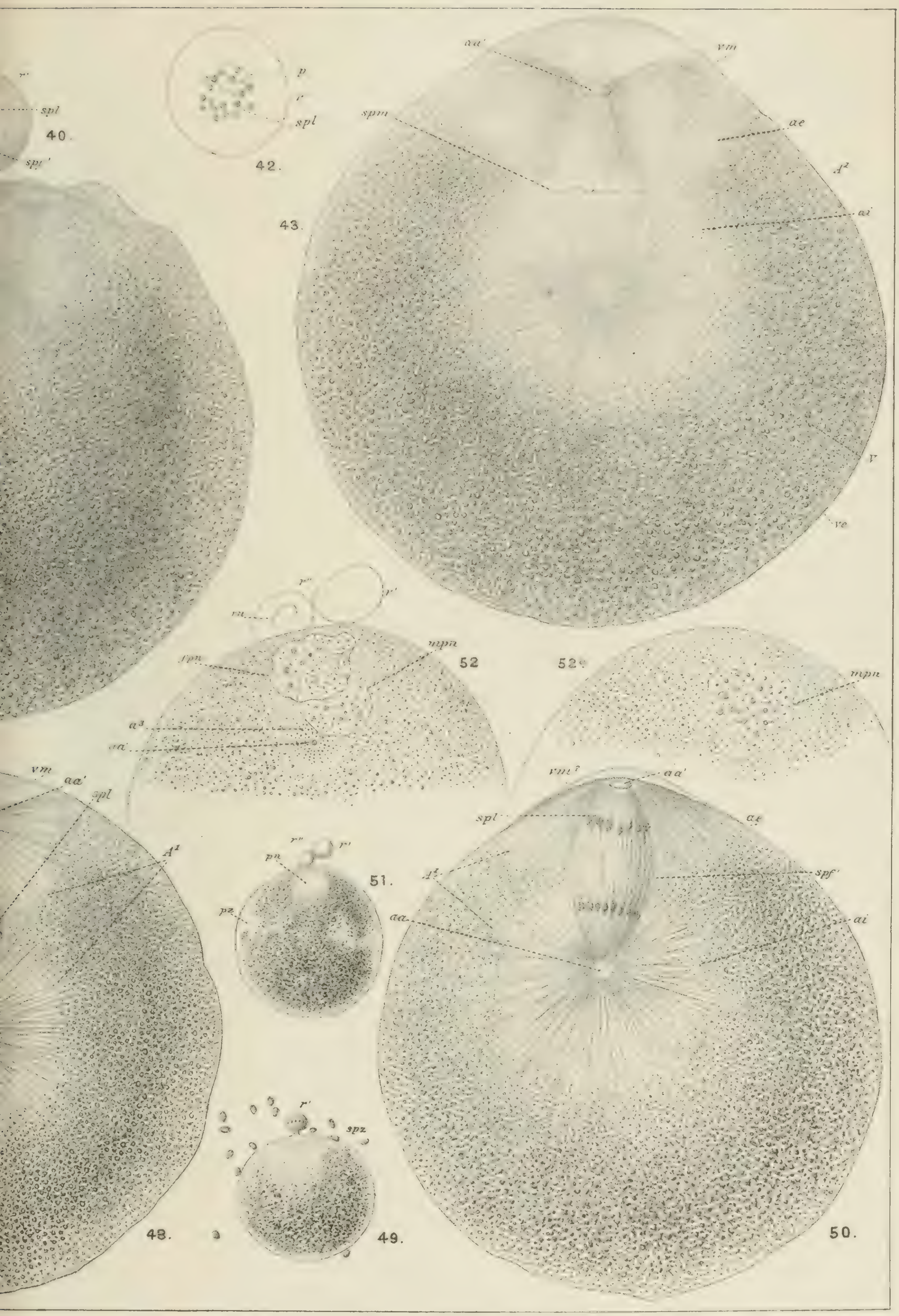

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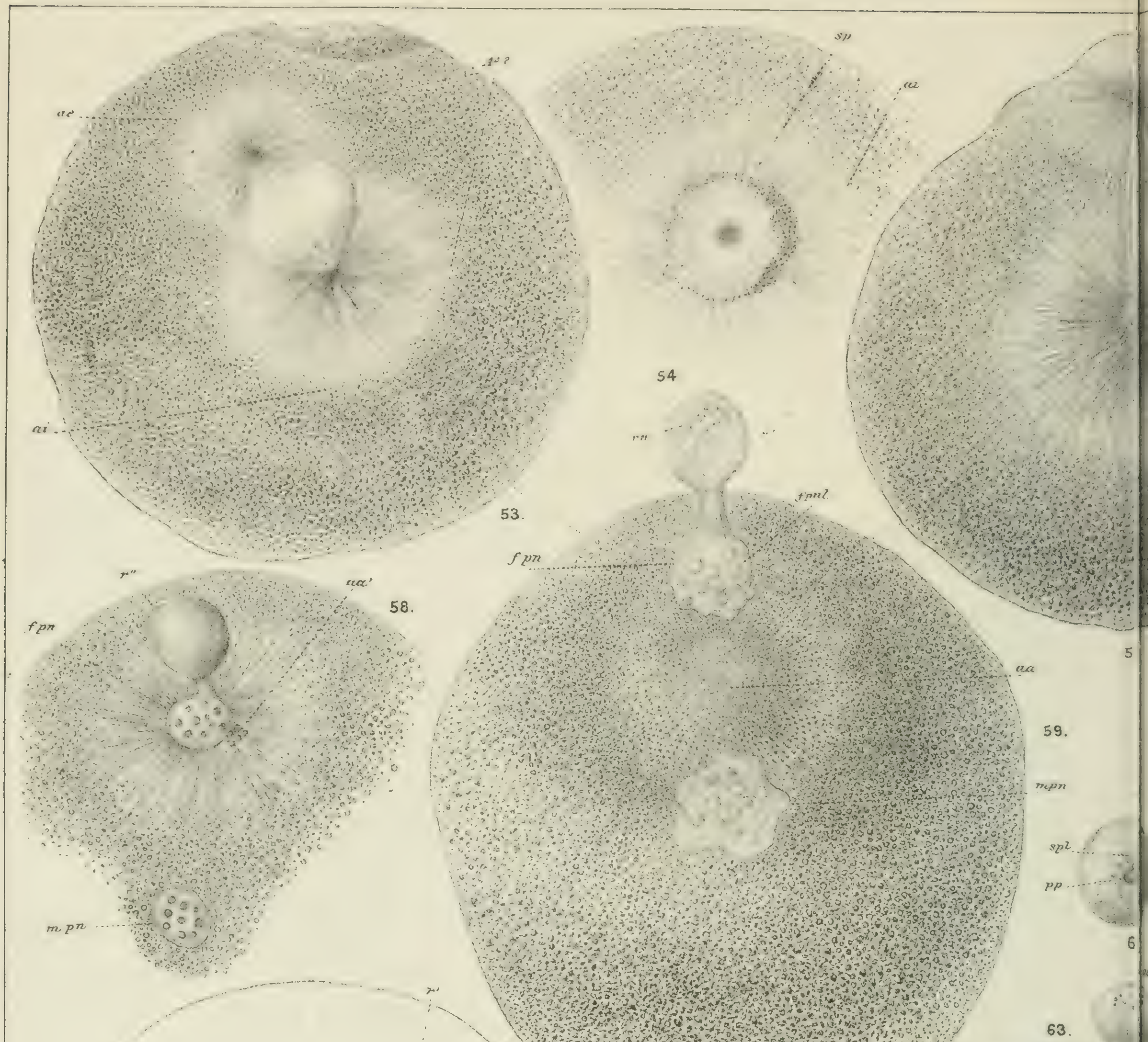
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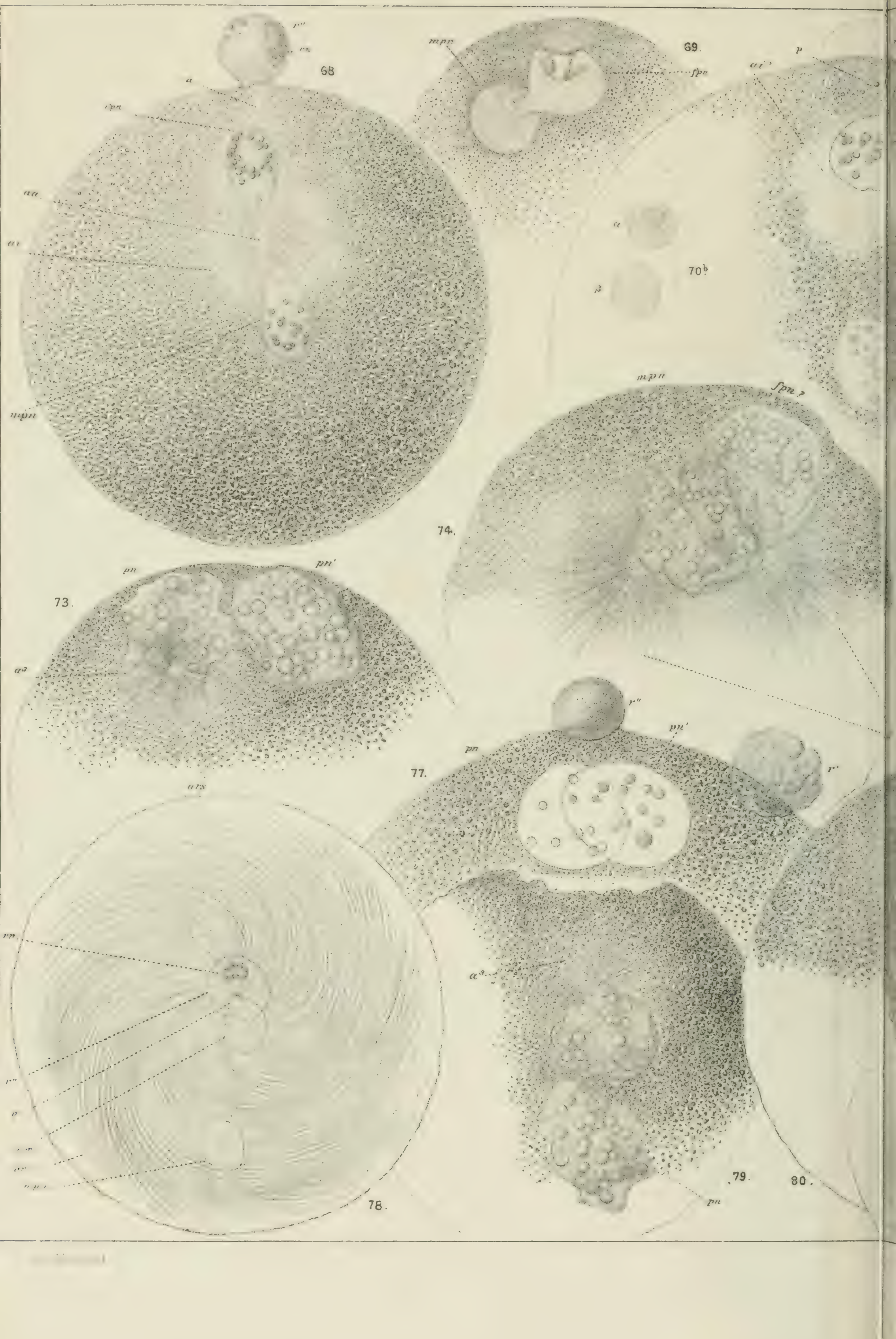
A Meisel lith.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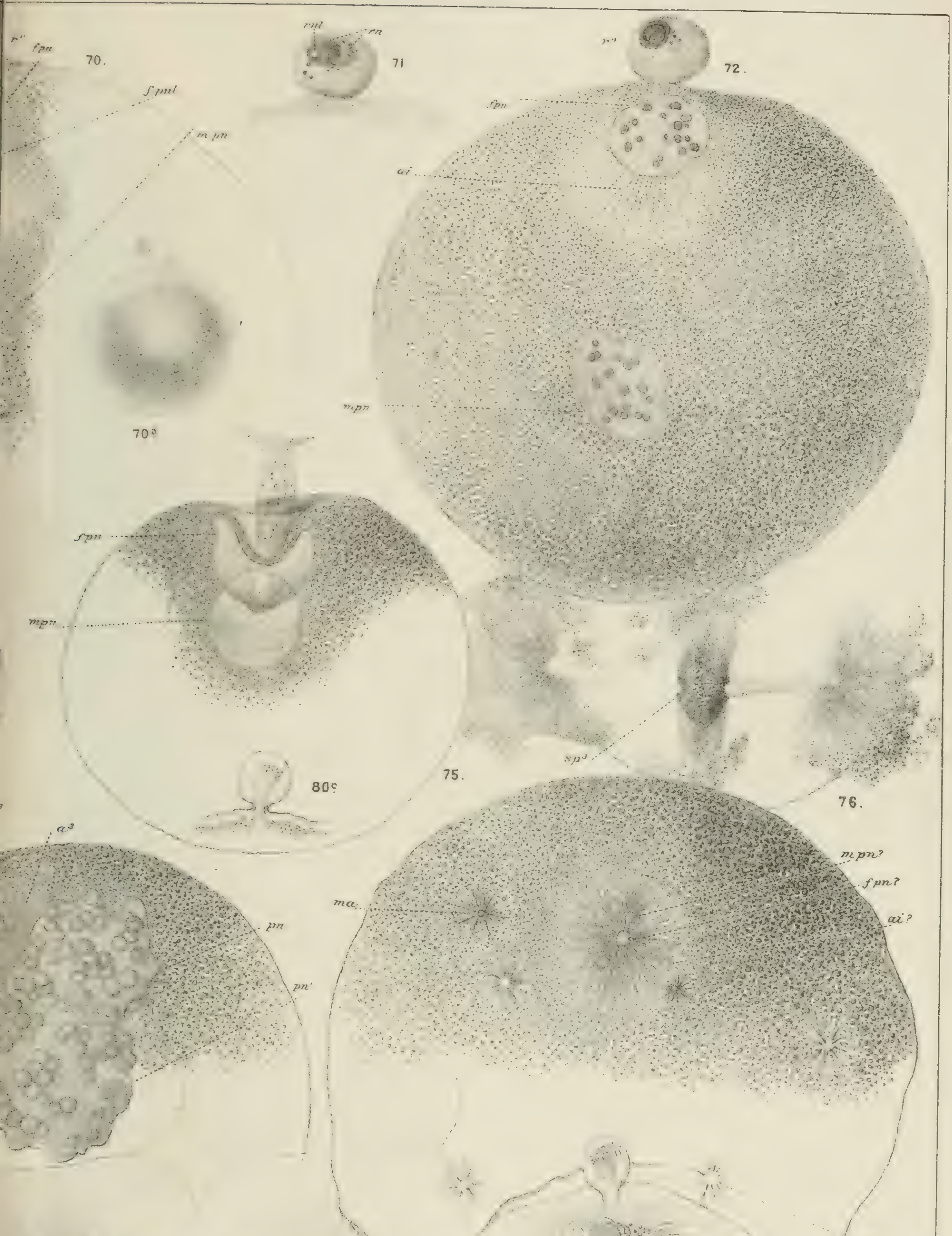
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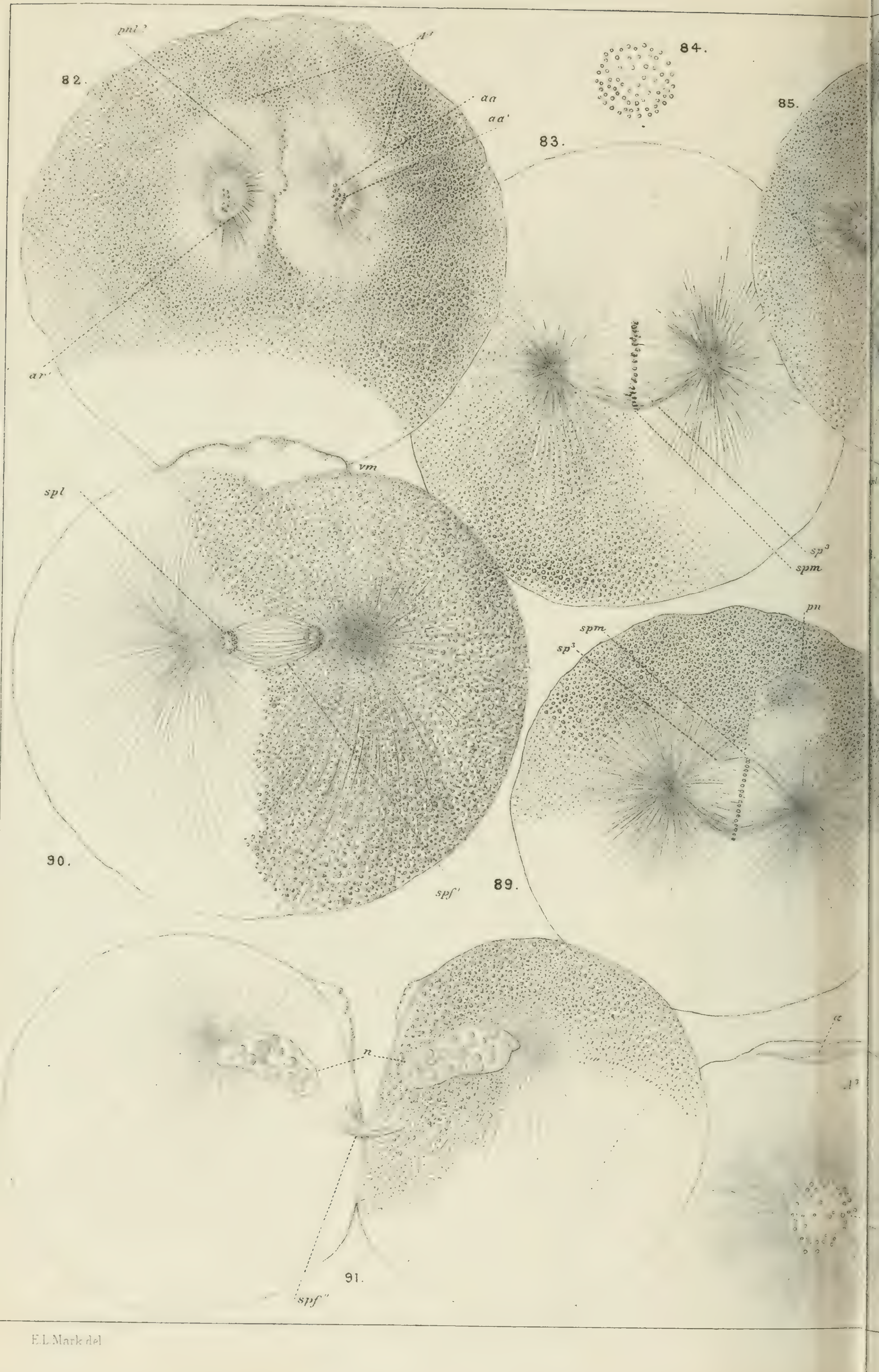
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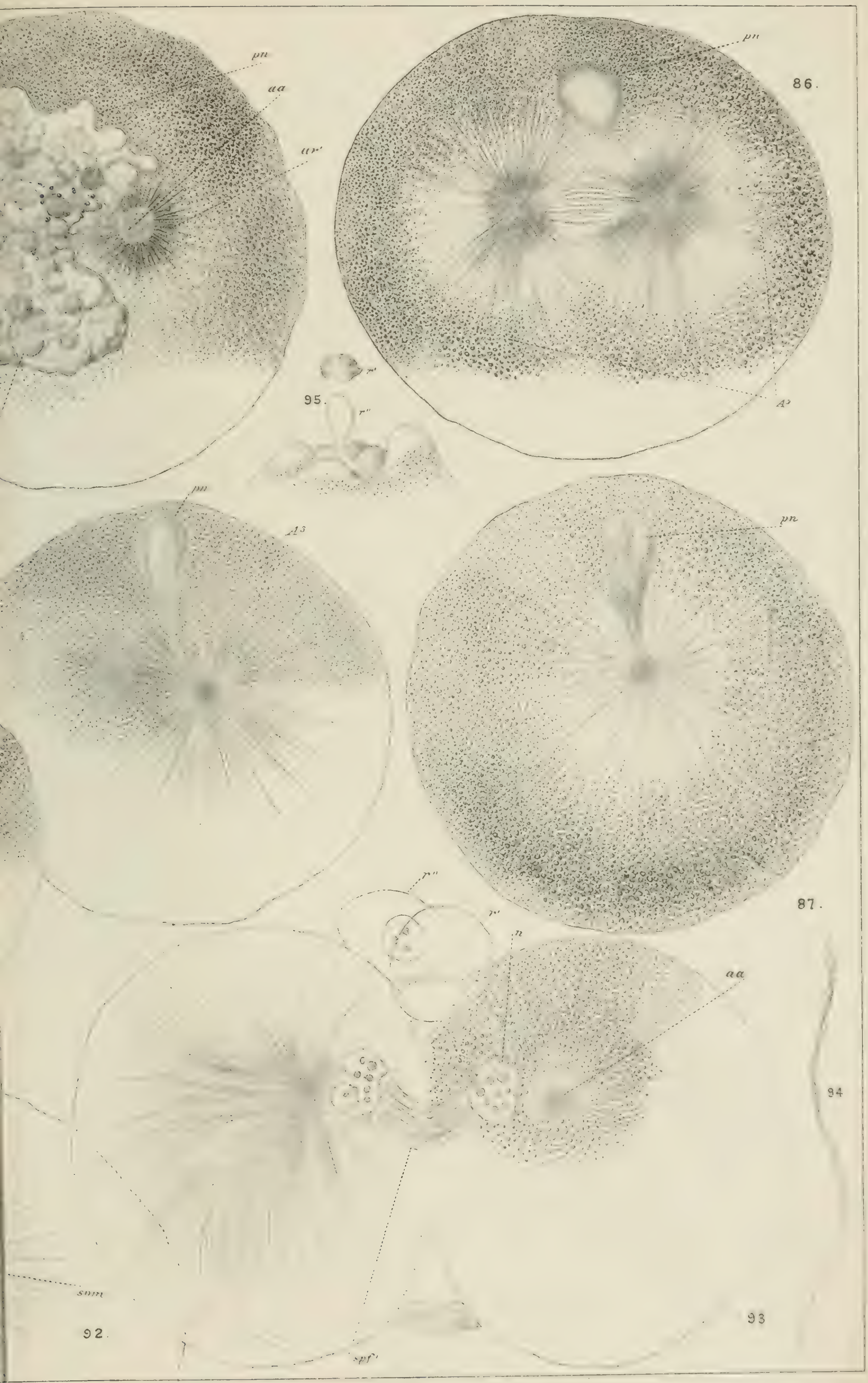
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